```
* * * * * * * * * *  Welcome to STN International  * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  FEB 28  PATDPAFULL - New display fields provide for legal status
                  data from INPADOC
 NEWS  4  FEB 28  BABS - Current-awareness alerts (SDIs) available
 NEWS  5  MAR 02  GBFULL: New full-text patent database on STN
 NEWS  6  MAR 03  REGISTRY/ZREGISTRY - Sequence annotations enhanced
 NEWS  7  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  8  MAR 22  KOREAPAT now updated monthly; patent information enhanced
 NEWS  9  MAR 22  Original IDE display format returns to REGISTRY/ZREGISTRY
 NEWS  10 MAR 22  PATDPASPC - New patent database available
 NEWS  11 MAR 22  REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  12 APR 04  EPFULL enhanced with additional patent information and new
                  fields
 NEWS  13 APR 04  EMBASE - Database reloaded and enhanced
 NEWS  14 APR 18  New CAS Information Use Policies available online
 NEWS  15 APR 25  Patent searching, including current-awareness alerts (SDIs),
                  based on application date in CA/CAplus and USPATFULL/USPAT2
                  may be affected by a change in filing date for U.S.
                  applications.
 NEWS  16 APR 28  Improved searching of U.S. Patent Classifications for
                  U.S. patent records in CA/CAplus
 NEWS  17 MAY 23  GBFULL enhanced with patent drawing images
 NEWS  18 MAY 23  REGISTRY has been enhanced with source information from
                  CHEMCATS
 NEWS  19 JUN 06  The Analysis Edition of STN Express with Discover!
                  (Version 8.0 for Windows) now available
 NEWS  20 JUN 13  RUSSIAPAT: New full-text patent database on STN
 NEWS  21 JUN 13  FRFULL enhanced with patent drawing images
 NEWS  22 JUN 27  MARPAT displays enhanced with expanded G-group definitions
                  and text labels
 NEWS  23 JUL 01  MEDICONF removed from STN
 NEWS  24 JUL 07  STN Patent Forums to be held in July 2005
 NEWS  25 JUL 13  SCISEARCH reloaded
 NEWS  26 JUL 20  Powerful new interactive analysis and visualization software,
                  STN AnaVist, now available

 NEWS EXPRESS  JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.42       0.42

FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Jul 2005 (20050721/PD)
FILE LAST UPDATED: 21 Jul 2005 (20050721/ED)
HIGHEST GRANTED PATENT NUMBER: US6920641
HIGHEST APPLICATION PUBLICATION NUMBER: US2005160510
CA INDEXING IS CURRENT THROUGH 21 Jul 2005 (20050721/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 21 Jul 2005 (20050721/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2005
```

USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2005

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATALL. Type FILE USPATALL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATALL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> s (filovir? or marburg or ebola)
891 FILOVIR?
1223 MARBURG
1104 EBOLA
L1 2139 (FILOVIR? OR MARBURG OR EBOLA)

=> s l1 and (gp1 or gp2)
854 GP1
742 GP2
L2 35 L1 AND (GP1 OR GP2)

=> d l2,cbib,1-35

L2 ANSWER 1 OF 35 USPATFULL on STN
2005:170843 Immunogenic minicells and methods of use.
Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
US 2005147590 A1 20050707
APPLICATION: US 2004-832000 A1 20040426 (10)
PRIORITY: US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DOCUMENT TYPE: Utility; APPLICATION.

L2 ANSWER 2 OF 35 USPATFULL on STN
2005:112372 Full-length human cDNAs encoding potentially secreted proteins.
Dumas Milne Edwards, Jean-Baptiste, Paris, FRANCE
Bougueleret, Lydie, Petit Lancy, SWITZERLAND
Jobert, Severin, Paris, FRANCE
US 2005096458 A1 20050505
APPLICATION: US 2003-643836 A1 20030819 (10)
PRIORITY: US 1999-169629P 19991208 (60)
US 2000-187470P 20000306 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 35 USPATFULL on STN
2005:68521 Process for making antifusogenic fusion peptides that form inclusion bodies.
Hoess, Eva, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Meier, Thomas, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Pestlin, Gabriele, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Popp, Friedrich, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Reichert, Klaus, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Schmuck, Rainer, Benediktbeuern, GERMANY, FEDERAL REPUBLIC OF
Schneidinger, Bernd, Hohenschaeftlarn/Neufahrn, GERMANY, FEDERAL REPUBLIC OF
Seidel, Christoph, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Tischer, Wilhelm, Peissenberg, GERMANY, FEDERAL REPUBLIC OF
US 2005058659 A1 20050317
APPLICATION: US 2004-969624 A1 20041020 (10)
PRIORITY: EP 2001-114497 20010615
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 35 USPATFULL on STN
2005:56119 Paramyxoviruses as gene transfer vectors to lung cells.
Pickles, Raymond, Chapel Hill, NC, UNITED STATES
Zhang, Liqun, Chapel Hill, NC, UNITED STATES
Peeples, Mark, Bexley, OH, UNITED STATES
Collins, Peter, Kensington, MD, UNITED STATES
Olsen, John, Chapel Hill, NC, UNITED STATES
US 2005048030 A1 20050303
APPLICATION: US 2004-492733 A1 20040727 (10)
WO 2002-US30813 20020927
PRIORITY: US 2001-326535P 20010928 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 5 OF 35 USPATFULL on STN
2005:43757 Recombinant influenza vectors with a PolII promoter and ribozymes for vaccines and gene therapy.
Kawaoka, Yoshihiro, Middleton, WI, UNITED STATES
Hamm, Stefan, River Vale, NJ, UNITED STATES
Ebihara, Hideki, Winnipeg, CANADA
US 2005037487 A1 20050217
APPLICATION: US 2004-855975 A1 20040527 (10)
PRIORITY: US 2003-473797P 20030528 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 6 OF 35 USPATFULL on STN
2004:328000 Development of a preventive vaccine for **filovirus** infection in primates.
Nabel, Gary, Washington, DC, UNITED STATES
Yang, Zhi-yong, Potomac, MD, UNITED STATES
Sullivan, Nancy, Kensington, MD, UNITED STATES
Sanchez, Anthony, Lilburn, GA, UNITED STATES
US 2004259825 A1 20041223
APPLICATION: US 2004-491121 A1 20040823 (10)
WO 2002-US30251 20020924
PRIORITY: US 2001-326476P 20011001 (60)
DOCUMENT TYPE: Utility; APPLICATION.

L2 ANSWER 7 OF 35 USPATFULL on STN
2004:315174 Compounds for the treatment of HIV infection.
Ernst, Justin T., San Diego, CA, UNITED STATES
Boman, Erik, Bonita, CA, UNITED STATES
Ceide, Susana C., San Diego, CA, UNITED STATES
Montalban, Antonio G., San Diego, CA, UNITED STATES
Nakanishi, Hiroshi, San Diego, CA, UNITED STATES
Roberts, Edward, San Diego, CA, UNITED STATES
Saiah, Eddine, La Jolla, CA, UNITED STATES
Lum, Christopher, San Diego, CA, UNITED STATES
Kemia, Inc. (U.S. corporation)
US 2004248850 A1 20041209
APPLICATION: US 2004-774040 A1 20040206 (10)
PRIORITY: US 2003-446713P 20030211 (60)
US 2003-523217P 20031118 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 8 OF 35 USPATFULL on STN
2004:292135 Method of inhibiting human metapneumovirus and human coronavirus in the prevention and treatment of severe acute respiratory syndrome (SARS).
Gallaher, William R., Pearl River, LA, UNITED STATES
Garry, Robert F., New Orleans, LA, UNITED STATES
US 2004229219 A1 20041118
APPLICATION: US 2004-834666 A1 20040429 (10)
PRIORITY: US 2003-466978P 20030430 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 9 OF 35 USPATFULL on STN
2004:282629 Inhibition of viral infection and spread with viral and RhoA-derived peptides.
Graham, Barney Scott, Nashville, TN, United States
Pastey, Manoj, Nashville, TN, United States
Vanderbilt University, Nashville, TN, United States (U.S. corporation)
US 6814968 B1 20041109
APPLICATION: US 1998-129565 19980805 (9)
PRIORITY: US 1998-87955P 19980604 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 10 OF 35 USPATFULL on STN
2004:280221 Novel nucleic acids and polypeptides.
Tang, Y. Tom, San Jose, CA, UNITED STATES
Wang, Zhiwei, Sunnyvale, CA, UNITED STATES
Weng, Gezhi, Piedmont, CA, UNITED STATES
Boyle, Bryan J., San Francisco, CA, UNITED STATES
Drmanac, Radoje T., Palo Alto, CA, UNITED STATES
US 2004219521 A1 20041104
APPLICATION: US 2002-128558 A1 20020422 (10)
PRIORITY: WO 2000-US35017 20001222
WO 2001-US2623 20010125
WO 2001-US3800 20010205
WO 2001-US4927 20010226
WO 2001-US4941 20010305
WO 2001-US8631 20010330
WO 2001-US8656 20010418
US 2001-339453P 20011211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 11 OF 35 USPATFULL on STN
2004:274281 Compositions and methods for treatment of neoplastic disease.
Terman, David S., Pebble Beach, CA, UNITED STATES
US 2004214783 A1 20041028
APPLICATION: US 2003-428817 A1 20030505 (10)
PRIORITY: US 2002-378988P 20020508 (60)
US 2002-389366P 20020615 (60)
US 2002-406697P 20020828 (60)
US 2002-406750P 20020829 (60)
US 2002-415310P 20021001 (60)
US 2002-415400P 20021002 (60)
US 2003-438686P 20030109 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 12 OF 35 USPATFULL on STN
2004:220839 Production of peptides in plants as viral coat protein fusions.
Palmer, Kenneth E., Vacaville, CA, UNITED STATES
Nguyen, Long V., Vacaville, CA, UNITED STATES
Toth, Rachel L., Fife, UNITED KINGDOM
Jones, Michael, Dundee, UNITED KINGDOM
Chapman, Sean, Fife, UNITED KINGDOM
Smolenska, Lisa, Dundee, UNITED KINGDOM
McCormick, Alison A., Vacaville, CA, UNITED STATES
Pogue, Gregory P., Vacaville, CA, UNITED STATES
US 2004170606 A1 20040902
APPLICATION: US 2003-654200 A1 20030903 (10)
PRIORITY: US 2002-386921P 20020607 (60)
US 2002-407795P 20020903 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 13 OF 35 USPATFULL on STN
2004:94257 Corona-virus-like particles comprising functionally deleted genomes.
Rottier, Petrus Josephus Marie, Groenekan, NETHERLANDS
Bosch, Berend-Jan, Utrecht, NETHERLANDS
US 2004071709 A1 20040415
APPLICATION: US 2003-414256 A1 20030414 (10)
PRIORITY: EP 2001-201861 20010517
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 14 OF 35 USPATFULL on STN
2004:70637 Monoclonal antibodies and complementarity-determining regions binding to **Ebola** glycoprotein.
Hart, Mary Kate, Frederick, MD, UNITED STATES
Wilson, Julie, Birmingham, AL, UNITED STATES
US 2004053865 A1 20040318
APPLICATION: US 2002-226795 A1 20020823 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 15 OF 35 USPATFULL on STN
2004:44495 Modifications of HIV Env, Gag, and Pol enhance immunogenicity for genetic immunization.
Nabel, Gary J., Washington, DC, UNITED STATES
Chakrabarti, Bimal K., Gaithersburg, MD, UNITED STATES
Huang, Yue, Gaithersburg, MD, UNITED STATES

US 2004033487 A1 20040219
APPLICATION: US 2003-359120 A1 20030204 (10)
PRIORITY: US 2001-279257P 20010328 (60)
US 2000-252115P 20001114 (60)
US 2000-225097P 20000814 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 16 OF 35 USPATFULL on STN
2004:38116 Composition and method for stimulating immune response to pathogen using complex adenoviral vector.
Wang, Danher, Mt. Pleasant, SC, UNITED STATES
US 2004028652 A1 20040212
APPLICATION: US 2002-327294 A1 20021219 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 17 OF 35 USPATFULL on STN
2003:318635 Novel nucleic acids and polypeptides.
Tang, Y. Tom, San Jose, CA, UNITED STATES
Yang, Yonghong, San Jose, CA, UNITED STATES
Wang, Zhiwei, Sunnyvale, CA, UNITED STATES
Weng, Gezhi, Piedmont, CA, UNITED STATES
Ma, Yunqing, Santa Clara, CA, UNITED STATES
US 2003224379 A1 20031204
APPLICATION: US 2002-243552 A1 20020912 (10)
PRIORITY: WO 2001-US2623 20010125
WO 2001-US3800 20010205
WO 2001-US4927 20010226
WO 2001-US4941 20010305
WO 2001-US8631 20010330
WO 2001-US8656 20010416
WO 2001-US14827 20010516
US 2001-322511P 20010913 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 18 OF 35 USPATFULL on STN
2003:318223 Expression of HIV polypeptides and production of virus-like particles.
Barnett, Susan, San Francisco, CA, UNITED STATES
Megede, Jan Zur, San Francisco, CA, UNITED STATES
Lian, Ying, Vallejo, CA, UNITED STATES
Hartog, Karin, Piedmont, CA, UNITED STATES
Liu, Hong, Castro Valley, CA, UNITED STATES
Greer, Catherine, Oakland, CA, UNITED STATES
Selby, Mark, Berkeley, CA, UNITED STATES
US 2003223964 A1 20031204
APPLICATION: US 2003-387336 A1 20030311 (10)
PRIORITY: US 1998-114495P 19981231 (60)
US 1999-168471P 19991201 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 19 OF 35 USPATFULL on STN
2003:311863 Multivalent vaccination using recombinant adenovirus.
Wang, Danher, Mt. Pleasant, SC, UNITED STATES
US 2003219458 A1 20031127
APPLICATION: US 2003-280915 A1 20030205 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 20 OF 35 USPATFULL on STN
2003:306361 **Filovirus** vectors and noninfectious **Filovirus**-based particles.
Kawaoka, Yoshihiro, Madison, WI, UNITED STATES
Jasenosky, Luke D., Madison, WI, UNITED STATES
Neumann, Gabriele, Madison, WI, UNITED STATES
US 2003215794 A1 20031120
APPLICATION: US 2003-353856 A1 20030129 (10)
PRIORITY: US 2002-353972P 20020131 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 21 OF 35 USPATFULL on STN
2003:268051 Monoclonal antibodies to **Ebola** glycoprotein.
Hart, Mary K., Frederick, MD, United States
Wilson, Julie A., Frederick, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army,

Washington, DC, United States (U.S. government)
US 6630144 B1 20031007
APPLICATION: US 2000-650086 20000829 (9)
PRIORITY: US 1999-151505P 19990830 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 22 OF 35 USPATFULL on STN
2003:219631 Full-length human cDNAs encoding potentially secreted proteins.
Dumas Milne Edwards, Jean-Baptiste, Paris, FRANCE
Bougueleret, Lydie, Petit Lancy, SWITZERLAND
Jobert, Severin, Paris, FRANCE
US 2003152921 A1 20030814
APPLICATION: US 2001-876997 A1 20010608 (9)
PRIORITY: US 1999-169629P 19991208 (60)
US 2000-187470P 20000306 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 23 OF 35 USPATFULL on STN
2003:209962 Expression of HIV polypeptides and production of virus-like particles.
Barnett, Susan W., San Francisco, CA, United States
Megede, Jan zur, San Francisco, CA, United States
Greer, Catherine, Oakland, CA, United States
Selby, Mark, San Francisco, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 6602705 B1 20030805
APPLICATION: US 1999-475515 19991230 (9)
PRIORITY: US 1998-114495P 19981231 (60)
US 1999-168471P 19991201 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 24 OF 35 USPATFULL on STN
2003:200475 Method of vaccination through serotype rotation.
Wang, Danher, Mt. Pleasant, SC, UNITED STATES
US 2003138459 A1 20030724
APPLICATION: US 2003-286332 A1 20030317 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 25 OF 35 USPATFULL on STN
2003:181681 End-locked five-helix protein.
Zhou, Genfa, Chestnut Hill, MA, UNITED STATES
US 2003125515 A1 20030703
APPLICATION: US 2002-193412 A1 20020711 (10)
PRIORITY: US 2001-304152P 20010711 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 26 OF 35 USPATFULL on STN
2003:169096 Nucleic acid sequences and expression system relating to Enterococcus faecium for diagnostics and therapeutics.
Doucette-Stamm, Lynn A., Framingham, MA, United States
Bush, David, Somerville, MA, United States
Genome Therapeutics Corporation, Waltham, MA, United States (U.S. corporation)
US 6583275 B1 20030624
APPLICATION: US 1998-107532 19980630 (9)
PRIORITY: US 1998-85598P 19980514 (60)
US 1997-51571P 19970702 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 27 OF 35 USPATFULL on STN
2003:158947 Chimeric **filovirus** glycoprotein.
Grogan, Case C., Gaithersburg, MD, UNITED STATES
Hevey, Michael C., Frederick, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2003108560 A1 20030612
APPLICATION: US 2002-66506 A1 20020131 (10)
PRIORITY: US 2001-267522P 20010131 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 28 OF 35 USPATFULL on STN
2003:152915 Process for making antifusogenic fusion peptides that form inclusion bodies.

Hoess, Eva, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Meier, Thomas, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Pestlin, Gabriele, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Popp, Friedrich, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Reichert, Klaus, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Schmuck, Rainer, Benediktbeuern, GERMANY, FEDERAL REPUBLIC OF
Schneidinger, Bernd, Hohenschaeftlarn/Neufahrn, GERMANY, FEDERAL REPUBLIC OF
Seidel, Christoph, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Tischer, Wilhelm, Peissenberg, GERMANY, FEDERAL REPUBLIC OF
US 2003104581 A1 20030605
APPLICATION: US 2002-158742 A1 20020530 (10)
PRIORITY: EP 2001-114497 20010615
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 29 OF 35 USPATFULL on STN
2003:95971 Adenovirus vector with multiple expression cassettes.
Wang, Danher, Mt. Pleasant, SC, United States
GenPhar, Inc., Mt. Pleasant, SC, United States (U.S. corporation)
US 6544780 B1 20030408
APPLICATION: US 2000-585599 20000602 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 30 OF 35 USPATFULL on STN
2003:50858 Recombinant influenza viruses for vaccines and gene therapy.
Kawaoka, Yoshihiro, Middleton, WI, UNITED STATES
Neumann, Gabriele, Nanuet, NY, UNITED STATES
US 2003035814 A1 20030220
APPLICATION: US 2001-971372 A1 20011004 (9)
PRIORITY: US 1999-127912P 19990406 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 31 OF 35 USPATFULL on STN
2002:279696 Genetic vaccine against human immunodeficiency virus.
Wang, Danher, Mt. Pleasant, SC, UNITED STATES
US 2002155127 A1 20021024
APPLICATION: US 2001-3035 A1 20011101 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 32 OF 35 USPATFULL on STN
2002:251079 Methods and compositions for the construction and use of envelope viruses as display particles.
Li, Min, Lutherville, MD, UNITED STATES
US 2002137022 A1 20020926
APPLICATION: US 2001-922503 A1 20010802 (9)
PRIORITY: US 2000-222697P 20000802 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 33 OF 35 USPATFULL on STN
2002:191539 Full-length human cDNAs encoding potentially secreted proteins.
Milne Edwards, Jean-Baptiste Dumas, Paris, FRANCE
Bougueleret, Lydie, Petit Lancy, SWITZERLAND
Jobert, Severin, Paris, FRANCE
US 2002102604 A1 20020801
APPLICATION: US 2000-731872 A1 20001207 (9)
PRIORITY: US 1999-169629P 19991208 (60)
US 2000-187470P 20000306 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 34 OF 35 USPATFULL on STN
2002:149128 Inhibitors of HIV membrane fusion.
Eckert, Debra M., Cambridge, MA, UNITED STATES
Chan, David C., Arcadia, CA, UNITED STATES
Malashkevich, Vladimir, Belmont, MA, UNITED STATES
Carr, Peter A., Somerville, MA, UNITED STATES
Kim, Peter S., Lexington, MA, UNITED STATES
Whitehead Institute For Biomedical Research, Cambridge, MA, UNITED STATES, 02142 (U.S. corporation)
US 2002077284 A1 20020620
APPLICATION: US 2000-746742 A1 20001221 (9)
PRIORITY: US 1997-43280P 19970417 (60)
US 1998-94676P 19980730 (60)
US 1998-100265P 19980914 (60)

US 1998-101058P 19980918 (60)
US 1999-132295P 19990503 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 35 OF 35 USPATFULL on STN
2001:211939 Human respiratory syncytial virus.
Zhao, Xun, Somerville, MA, United States
Singh, Mona, Princeton, NJ, United States
Malashkevich, Vladimir, Belmont, MA, United States
Kim, Peter S., Lexington, MA, United States
Whitehead Institute for Biomedical Research, Cambridge, MA, United States, 02142 (U.S. corporation)
US 2001043931 A1 20011122
APPLICATION: US 2000-730504 A1 20001205 (9)
PRIORITY: US 1999-169327P 19991206 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l2,cbib,ab,clm,21

L2 ANSWER 21 OF 35 USPATFULL on STN
2003:268051 Monoclonal antibodies to **Ebola** glycoprotein.
Hart, Mary K., Frederick, MD, United States
Wilson, Julie A., Frederick, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6630144 B1 20031007
APPLICATION: US 2000-650086 20000829 (9)
PRIORITY: US 1999-151505P 19990830 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In this application are described **Ebola** GP monoclonal antibodies and epitopes recognized by these monoclonal antibodies. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention, and/or therapeutical treatment of **Ebola** virus infections in vitro and in vivo.

CLM What is claimed is:

1. An isolated monoclonal antibody which recognizes **Ebola** virus GP, wherein the epitope that binds or is recognized by said antibody is within SEQ ID NO:6 or SEQ ID NO:8.

2. The antibody according to claim 1, wherein the antibody binds **Ebola** virus in vitro.

3. The antibody according to claim 1, wherein the antibody immunoprecipitates GP from supernatants or cell lysates of cell cultures infected with **Ebola** virus.

4. The antibody according to claim 1 wherein said epitope is within SEQ ID NO:6, and is further within SEQ ID NO:7.

5. The antibody according to claim 1 wherein said epitope is within SEQ ID NO:8, and is further within SEQ ID NO:9.

6. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 13F6-1-2 with Accession no. PTA-373.

7. An antibody which competes with the antibody of claim 6 for binding to **Ebola** virus GP.

8. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 6D3-1-1 with Accession no. PTA-374.

9. An antibody which competes with the antibody of claim 8 for binding to **Ebola** virus GP.

10. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 13C6-1-1 with Accession no. PTA-375.

11. An antibody which competes with the antibody of claim 10 for binding to **Ebola** virus GP.

12. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 6D8-1-2 with Accession no. PTA-376.

13. An antibody which competes with the antibody of claim 12 for binding to **Ebola** virus GP.

14. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 12B5-1-1 with Accession no. PTA-436.

15. An antibody which competes with the antibody of claim 14 for binding to **Ebola** virus GP.

16. A mixture comprising **Ebola** virus antibodies comprising one or more antibodies selected from the group consisting of an antibody produced by hybridoma EGP 13F6-1-2 accession no. PTA 373; an antibody produced by hybridoma EGP 6D3-1-1 accession no. PTA 374; an antibody produced by hybridoma EGP 13C6-1-1 accession no. PTA 375; an antibody produced by hybridoma EGP 6D8-1-2 accession no. PTA 376; and an antibody produced by hybridoma EGP 12B5-1-1 accession no. PTA 436.

17. A monoclonal antibody producing cell line that produces a monoclonal antibody according to claim 1.

18. The cell line according to claim 17, selected from the group consisting of cell line EGP 13F6-1-2 (ATCC accession no. PTA 373), cell line EGP 6D3-1-1 (ATCC accession no. PTA 374), cell line EGP 13C6-1-1 (ATCC accession no. PTA 375), cell line EGP 6D8-1-2 (ATCC accession no. PTA 376), and cell line EGP 12B5-1-1 (ATCC accession no. PTA 436).

19. An antiidiotypic antibody produced from any of the monoclonal antibodies selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

20. An antiidiotypic antibody produced from an antibody which competes for binding to GP with an antibody selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

```
=> d his

     (FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005)

     FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
L1          2139 S (FILOVIR? OR MARBURG OR EBOLA)
L2            35 S L1 AND (GP1 OR GP2)

=> s l1 and (bivalent or multivalent)
          18249 BIVALENT
          13053 MULTIVALENT
L3          255 L1 AND (BIVALENT OR MULTIVALENT)

=> s l3 and (marburg/ti or ebola/ti or filovir?/ti)
              3 MARBURG/TI
              6 EBOLA/TI
              7 FILOVIR?/TI
L4            8 L3 AND (MARBURG/TI OR EBOLA/TI OR FILOVIR?/TI)

=> s l4 not l2
L5            6 L4 NOT L2

=> d l5,cbib,1-6

L5   ANSWER 1 OF 6  USPATFULL on STN
2004:190090 Ebola virion proteins expressed from venezuelan equine
    encephalitis (VEE) virus replicons.
    Hart, Mary K., Frederick, MD, UNITED STATES
    Wilson, Julie A., Frederick, MD, UNITED STATES
    Pushko, Peter, Frederick, MD, UNITED STATES
    Smith, Jonathan F., Sabillasville, MD, UNITED STATES
    Schmaljohn, Alan L., Frederick, MD, UNITED STATES
    US 2004146859 A1 20040729
    APPLICATION: US 2003-696633  A1  20031029 (10)
    PRIORITY: US 1998-91403P  19980629 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5   ANSWER 2 OF 6  USPATFULL on STN
2004:76190 Generation of virus-like particles and demonstration of lipid rafts
    as sites of filovirus entry and budding.
    Bavari, Sina, Frederick, MD, UNITED STATES
    Aman, M. Javad, Potomac, MD, UNITED STATES
```

Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2004057967 A1 20040325
APPLICATION: US 2002-289839 A1 20021107 (10)
PRIORITY: US 2001-338936P 20011107 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 6 USPATFULL on STN
2003:318273 **Ebola** peptides and immunogenic compositions containing same.
Hart, Mary Katherine, Frederick, MD, UNITED STATES
Wilson, Julie Ann, Birmingham, AL, UNITED STATES
Olinger, Gene Garrard, JR., Frederick, MD, UNITED STATES
Bailey, Michael Adam, Frederick, MD, UNITED STATES
US 2003224015 A1 20031204
APPLICATION: US 2003-384976 A1 20030310 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 6 USPATFULL on STN
2003:219300 **Marburg** virus vaccines.
Hevey, Michael C., Frederick, MD, UNITED STATES
Negley, Diane L., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2003152590 A1 20030814
APPLICATION: US 2002-267322 A1 20021009 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 6 USPATFULL on STN
2003:40417 **Marburg** virus vaccines.
Hevey, Michael C., Frederick, MD, United States
Negley, Diane L., Frederick, MD, United States
Pushko, Peter, Frederick, MD, United States
Smith, Jonathan F., Sabillasville, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6517842 B1 20030211
APPLICATION: US 1999-336910 19990621 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 6 USPATFULL on STN
2002:294539 **EBOLA** VIRION PROTEINS EXPRESSED FROM VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS REPLICONS.
HART, MARY K., FREDERICK, MD, UNITED STATES
WILSON, JULIE A., FREDERICK, MD, UNITED STATES
PUSHKO, PETER, FREDERICK, MD, UNITED STATES
SMITH, JONATHAN F., SABILLASVILLE, MD, UNITED STATES
SCHMALJOHN, ALAN L., FREDERICK, MD, UNITED STATES
US 2002164582 A1 20021107
APPLICATION: US 1999-337946 A1 19990622 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l5,cbib,ab,clm,1,3-6

L5 ANSWER 1 OF 6 USPATFULL on STN
2004:190090 **Ebola** virion proteins expressed from venezuelan equine encephalitis (VEE) virus replicons.
Hart, Mary K., Frederick, MD, UNITED STATES
Wilson, Julie A., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2004146859 A1 20040729
APPLICATION: US 2003-696633 A1 20031029 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Using the **Ebola** GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is

protective against infection with **Ebola** virus is described.

CLM What is claimed is:

1. A DNA fragment which encodes a GP **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:1, or a polynucleotide fragment comprising at least 15 nucleotides.

2. A DNA fragment which encodes a NP **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:2, or a polynucleotide fragment comprising at least 15 nucleotides.

3. A DNA fragment which encodes a VP24 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:3, or a polynucleotide fragment comprising at least 15 nucleotides.

4. A DNA fragment which encodes a VP30 **Ebola** protein, said DNA fragment comprising the sequence specified in any of SEQ ID NO:4 and SEQ ID NO:7, or a polynucleotide fragment comprising at least 15 nucleotides.

5. A DNA fragment which encodes a VP35 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:5, or a polynucleotide fragment comprising at least 15 nucleotides.

6. A DNA fragment which encodes a VP40 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:6, or a polynucleotide fragment comprising at least 15 nucleotides.

7. A DNA fragment which encodes a GP **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:17 or a conservative substitution thereof.

8. A DNA fragment which encodes a NP **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:18 or a conservative substitution thereof.

9. A DNA fragment which encodes a VP24 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:19 or a conservative substitution thereof.

10. A DNA fragment which encodes a VP30 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in any of SEQ ID NO:20 and SEQ ID NO:23 or a conservative substitution thereof.

11. A DNA fragment which encodes a VP35 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:21 or a conservative substitution thereof.

12. A DNA fragment which encodes a VP40 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:22 or a conservative substitution thereof.

13. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the **Ebola** virus DNA fragments chosen from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6 and 7 or a fragment thereof comprising at least 15 nucleotides.

14. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the **Ebola** virus DNA fragments chosen from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24 and 25 or a conservative substitution thereof.

15. The recombinant DNA construct of claim 13 wherein said DNA fragment induces a cytotoxic T lymphocyte, response or antibody response.

16. The recombinant DNA construct of claim 14 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

17. A recombinant DNA construct according to claim 13 wherein said vector is an expression vector.

18. A recombinant DNA construct according to claim 13 wherein said vector is a prokaryotic vector.

19. A recombinant DNA construct according to claim 13 wherein said vector is a eukaryotic vector.

20. A recombinant DNA construct according to claim 14 wherein said vector is an expression vector.

21. A recombinant DNA construct according to claim 14 wherein said vector is a prokaryotic vector.

22. A recombinant DNA construct according to claim 14 wherein said vector is a eukaryotic vector.

23. The recombinant DNA construct of claim 17 wherein said vector is a VEE virus replicon vector.

24. The recombinant DNA construct of claim 20 wherein said vector is a VEE virus replicon vector.

25. The recombinant DNA construct according to claim 23 wherein said **Ebola** virus DNA fragments are from **Ebola** Zaire 1976.

26. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP24.

27. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP30.

28. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP35.

29. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP40.

30. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboNP.

31. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboGP.

32. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboVP30(#2).

33. Self replicating RNA produced from a construct chosen from the group consisting of EboVP24ReP, EboVP30ReP, EboVP35ReP, EboVP40ReP, EboVPNPReP, EboVPGPReP, and EboVP30ReP(#2).

34. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 33.

35. A pharmaceutical composition comprising infectious alphavirus particles according to claim 34 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

36. A host cell transformed with a recombinant DNA construct-according to claim 13.

37. A host cell transformed with a recombinant DNA construct according to claim 14.

38. A host cell according to claim 36 wherein said host cell is prokaryotic.

39. A host cell according to claim 36 wherein said host cell is eukaryotic.

40. A host cell according to claim 37 wherein said host cell is prokaryotic.

41. A host cell according to claim 37 wherein said host cell is eukaryotic.

42. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 36 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

43. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 37 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

44. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 38 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

45. A method for producing **Ebola** virus proteins comprising culturing

the cells according to claim 39 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

46. An isolated and purified **Ebola** GP protein specified in SEQ ID NO:17 and conservative substitutions thereof, or an immunologically identifiable portion thereof.

47. An isolated and purified **Ebola** NP protein specified in SEQ ID NO:18 and conservative substitutions thereof or an immunologically identifiable portion thereof.

48. An isolated and purified **Ebola** VP24 protein specified in SEQ ID NO:19 and conservative substitutions thereof or an immunologically identifiable portion thereof.

49. An isolated and purified **Ebola** VP30 protein specified in any of SEQ ID NO:20 and SEQ ID NO:23 and conservative substitutions thereof or an immunologically identifiable portion thereof.

50. An isolated and purified **Ebola** VP35 protein specified in SEQ ID NO:21 and conservative substitutions thereof or an immunologically identifiable portion thereof.

51. An isolated and purified **Ebola** VP40 protein specified in SEQ ID NO:22 and conservative substitutions thereof or an immunologically identifiable portion thereof.

52. An antibody to a peptide encoded by the sequence specified in SEQ ID NO:17, 18, 19, 20, 21, 22, 23, 24, and 25.

53. A method for detecting **Ebola** virus infection comprising contacting a sample from a subject suspected of having **Ebola** virus infection with a antibody according to claim 52 and detecting the presence or absence by detecting the presence or absence of a complex formed between the **Ebola** protein and antibodies specific therefor.

54. A method for detecting the presence or absence of **Ebola** virus GP RNA in a sample using the polymerase chain reaction using primers for **Ebola** GP nucleic acid sequence specified in SEQ ID NO:1 for GP.

55. An **Ebola** infection diagnostic kit comprising at least 12 consecutive nucleotides of SEQ ID NO:1 specific for the amplification of DNA or RNA of **Ebola** virus in a sample using the polymerase chain reaction and ancillary reagents suitable for use in such a reaction for detecting the presence or absence of **Ebola** virus DNA or RNA in a sample.

56. A vaccine for **Ebola** comprising alphavirus particles of claim 34.

57. A method for the diagnosis of **Ebola** virus infection comprising the steps of: (i) contacting a sample from an individual suspected of having **Ebola** virus infection with an antibody to **Ebola** proteins according to claim 52; and (ii) detecting the presence or absence of **Ebola** virus infection by detecting the presence or absence of a complex formed between **Ebola** proteins and antibodies specific therefor.

58. A pharmaceutical composition comprising the self replicating RNA of claim 33 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

59. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of VRepEboVP24, VRepEboVP30, VRepEboVP35, VRepEboVP40, VRepEboNP, VRepEboGP, and VRepEboVP30(#2), in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier and/or adjuvant.

60. A pharmaceutical composition comprising comprising a peptide encoded by any of SEQ ID NO:24 and SEQ ID NO:25, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

L5 ANSWER 3 OF 6 USPATFULL on STN
2003:318273 **Ebola** peptides and immunogenic compositions containing same.
Hart, Mary Katherine, Frederick, MD, UNITED STATES
Wilson, Julie Ann, Birmingham, AL, UNITED STATES
Olinger, Gene Garrard, JR., Frederick, MD, UNITED STATES
Bailey, Michael Adam, Frederick, MD, UNITED STATES
US 2003224015 A1 20031204
APPLICATION: US 2003-384976 A1 20030310 (10)

PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Using CTL epitopes to the **Ebola** GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is protective against infection with **Ebola** virus is described.

CLM What is claimed is:

1. An isolated GP **Ebola** peptide comprising the sequence specified in SEQ ID NO:29, or a peptide fragment comprising at least 9 consecutive amino acids.

2. The peptide of claim 1, wherein the peptide fragment has the sequence of YFGPAAEGI (SEQ ID NO:42).

3. An isolated NP **Ebola** peptide comprising the sequence specified in SEQ ID NO:24.

4. An isolated NP **Ebola** peptide comprising the sequence specified in SEQ ID NO:26, SEQ ID NO:27 or SEQ ID NO:28.

5. An isolated VP24 **Ebola** peptide comprising the sequence specified in SEQ ID NO:25, or a peptide fragment comprising at least 9 consecutive amino acids.

6. The peptide of claim 5, wherein the peptide fragment has the sequence of KFINKLDAL (SEQ ID NO:43).

7. An isolated VP24 **Ebola** peptide comprising the sequence specified in SEQ ID NO:30, or a peptide fragment comprising at least 9 consecutive amino acids.

8. The peptide of claim 7, wherein the peptide fragment has the sequence of NYNGLLSSI (SEQ ID NO:44).

9. An isolated VP24 **Ebola** peptide comprising the sequence specified in SEQ ID NO:31, or a peptide fragment comprising at least 9 consecutive amino acids.

10. The peptide of claim 9, wherein the peptide fragment has the sequence of PGPAKFSLL (SEQ ID NO:45).

11. An isolated VP30 **Ebola** peptide comprising the sequence specified in SEQ ID NO:32, or a peptide fragment comprising at least 9 consecutive amino acids.

12. The peptide of claim 11, wherein the peptide fragment has the sequence of LSLLCETHLR (SEQ ID NO:46).

13. An isolated VP30 **Ebola** peptide comprising the sequence specified in SEQ ID NO:33, or a peptide fragment comprising at least 9 consecutive amino acids.

14. The peptide of claim 13, wherein the peptide fragment has the sequence of MFITAFLNI (SEQ ID NO:47).

15. An isolated VP35 **Ebola** peptide comprising the sequence specified in SEQ ID NO:34, SEQ ID NO:35 or SEQ ID NO:36.

16. An isolated VP40 **Ebola** peptide comprising the sequence specified in SEQ ID NO:37, or a peptide fragment comprising at least 9 consecutive amino acids.

17. The peptide of claim 16, wherein the peptide fragment has the sequence of EFVLPPVQL (SEQ ID NO:48).

18. An isolated VP40 **Ebola** peptide comprising the sequence specified in SEQ ID NO:38, or a peptide fragment comprising at least 6 consecutive amino acids.

19. The peptide of claim 18, wherein the peptide fragment has the sequence of FLVPPV (SEQ ID NO:49) or QYFTFDLTALK (SEQ ID NO:50).

20. An isolated VP40 **Ebola** peptide comprising the sequence specified in SEQ ID NO:39, or a peptide fragment comprising at least 9 consecutive amino acids.

21. The peptide of claim 20, wherein the peptide fragment has the sequence of TSPEKIQAI (SEQ ID NO:51).

22. An isolated VP40 **Ebola** peptide comprising the sequence specified in SEQ ID NO:40, or a peptide fragment comprising at least 8 consecutive amino acids.

23. The peptide of claim 22, wherein the peptide fragment has the sequence of RIGNQAFL (SEQ ID NO:52).

24. An isolated VP40 **Ebola** peptide comprising the sequence specified in SEQ ID NO:41, or a peptide fragment comprising at least 8 consecutive amino acids.

25. The peptide of claim 24, wherein the peptide fragment has the sequence of QAFLQEFV (SEQ ID NO:53).

26. An isolated DNA fragment which encodes the GP **Ebola** peptide of claim 1.

27. A DNA fragment which encodes the NP **Ebola** peptide of claim3.

28. A DNA fragment which encodes the SEQ ID NO:26 NP **Ebola** peptide of claim 4.

29. A DNA fragment which encodes the SEQ ID NO:27 NP **Ebola** peptide of claim 4.

30. A DNA fragment which encodes the SEQ ID NO:28 NP **Ebola** peptide of claim 4.

31. A DNA fragment which encodes the VP24 **Ebola** peptide of claim 5.

32. A DNA fragment which encodes the VP24 **Ebola** peptide of claim 7.

33. A DNA fragment which encodes the VP24 **Ebola** peptide of claim 9.

34. A DNA fragment which encodes the VP30 **Ebola** peptide of claim 11.

35. A DNA fragment which encodes the VP30 **Ebola** peptide of claim 13.

36. A DNA fragment which encodes the SEQ ID NO:34 VP35 **Ebola** peptide of claim 15.

37. A DNA fragment which encodes the SEQ ID NO:35 VP35 **Ebola** peptide of claim 15.

38. A DNA fragment which encodes the SEQ ID NO:36 VP35 **Ebola** peptide of claim 15.

39. A DNA fragment which encodes the VP40 **Ebola** peptide of claim 16.

40. A DNA fragment which encodes the VP40 **Ebola** peptide of claim 18.

41. A DNA fragment which encodes the VP40 **Ebola** peptide of claim 20.

42. A DNA fragment which encodes the VP40 **Ebola** peptide of claim 22.

43. A DNA fragment which encodes the VP40 **Ebola** peptide of claim 24.

44. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the **Ebola** virus DNA fragments encoding a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53.

45. The recombinant DNA construct of claim 44, wherein the DNA fragment encodes the peptide of SEQ ID NO:24 or SEQ ID NO:25.

46. The recombinant DNA construct of claim 44 wherein said DNA fragment induces a cytotoxic T lymphocyte response.

47. The recombinant DNA construct according to claim 44 wherein said vector is an expression vector.

48. The recombinant DNA construct according to claim 44 wherein said vector is a VEE virus replicon vector.

49. The recombinant DNA construct according to claim 44 wherein said vector is a eukaryotic vector.

50. The recombinant DNA construct of claim 44 wherein said vector is selected from the group consisting of Venezuelan Equine Encephalitis (VEE) virus replicon vector, eastern equine encephalitis virus replicon vector, western equine encephalitis virus replicon vector, Semliki forest virus replicon vector and Sindbis virus replicon vector.

51. A pharmaceutical composition comprising SEQ ID NO:24, SEQ ID NO:25, or both.

52. A pharmaceutical composition comprising a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

53. A vaccine against **Ebola** infection comprising a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

54. A vaccine against **Ebola** infection comprising SEQ ID NO:24, SEQ ID NO:25, or both, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

55. A vaccine against **Ebola** infection comprising virus replicon particles expressing at least one of the peptides specified by SEQ ID NOs:24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

56. The vaccine of claim 55, wherein the virus replicon particles are produced from a replicon vector selected from the group consisting of Venezuelan Equine Encephalitis (VEE) virus, eastern equine encephalitis, western equine encephalitis, Semliki forest and Sindbis.

57. A vaccine against **Ebola** infection comprising virus replicon particles expressing at least one of the peptides specified by SEQ ID NOs:24 and 25, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

58. The vaccine of claim 57, wherein the virus replicon particles are produced from a replicon vector selected from the group consisting of Venezuelan Equine Encephalitis (VEE) virus, eastern equine encephalitis, western equine encephalitis, Semliki forest and Sindbis.

59. A method for inducing in a mammal a cytotoxic T lymphocyte response to an **Ebola** peptide comprising the step of: administering to a mammal an immunogenic composition comprising a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant, under such conditions that the peptide induces a protective cytotoxic T lymphocyte response.

60. A method for inducing in a mammal a cytotoxic T lymphocyte response to an **Ebola** peptide comprising the step of: administering to a mammal an immunogenic composition comprising a peptide selected from the group consisting of SEQ ID NOs:24 and 25, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant, under such conditions that the peptide induces a protective cytotoxic T lymphocyte response.

61. A method for inducing in a mammal a cytotoxic T lymphocyte response to an **Ebola** peptide comprising the step of: administering to a mammal a recombinant DNA construct that expresses a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant, under such conditions that the peptide induces a protective cytotoxic T lymphocyte response.

62. The method of claim 61, wherein the recombinant DNA construct comprises: (i) a replicon vector selected from the group consisting of Venezuelan Equine Encephalitis (VEE) virus, eastern equine encephalitis, western equine encephalitis, Semliki forest and Sindbis, and (ii) at

least one of the **Ebola** virus DNA fragments encoding a peptide selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 and 53, and mixtures thereof.

63. A method for inducing in a mammal a cytotoxic T lymphocyte response to an **Ebola** peptide comprising the step of: administering to a mammal a recombinant DNA construct that expresses a peptide selected from the group consisting of SEQ ID NOs:24 and 25, and mixtures thereof, in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant, under such conditions that the peptide induces a protective cytotoxic T lymphocyte response.

64. The method of claim 63, wherein the recombinant DNA construct comprises: (i) a replicon vector selected from the group consisting of Venezuelan Equine Encephalitis (VEE) virus, eastern equine encephalitis, western equine encephalitis, Semliki forest and Sindbis, and (ii) at least one of the **Ebola** virus DNA fragments encoding a peptide selected from the group consisting of SEQ ID NOs: 24 and 25, and mixtures thereof.

65. An immunogenic composition comprising **Ebola** peptides VP30, VP35, and VP40.

66. The immunogenic composition of claim 65 wherein the VP30 peptide has the amino acid sequence of SEQ ID NO:20.

67. The immunogenic composition of claim 65 wherein the VP35 peptide has the amino acid sequence of SEQ ID NO:21

68. The immunogenic composition of claim 65 wherein the VP40 peptide has the amino acid sequence of SEQ ID NO:22.

69. The immunogenic composition of claim 65 wherein the VP30 peptide has the amino acid sequence of SEQ ID NO:20, the VP35 peptide has the amino acid sequence of SEQ ID NO:21, and the VP40 peptide has the amino acid sequence of SEQ ID NO:22.

L5 ANSWER 4 OF 6 USPATFULL on STN
2003:219300 **Marburg** virus vaccines.
Hevey, Michael C., Frederick, MD, UNITED STATES
Negley, Diane L., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2003152590 A1 20030814
APPLICATION: US 2002-267322 A1 20021009 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Using the MBGV GP, NP, and virion proteins, a method and composition for use in inducing an immune response which is protective against infection with MBGV in nonhuman primates is described.
CLM What is claimed is:
1. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the MBGV virus DNA fragments encoding any one of GP, NP, VP40, VP35, VP30, VP24, and GPΔTM.

2. A recombinant DNA construct according to claim 1 wherein said vector is an expression vector.

3. A recombinant DNA construct according to claim 1 wherein said vector is a prokaryotic vector.

4. A recombinant DNA construct according to claim 1 wherein said vector is a eukaryotic vector.

5. The recombinant DNA construct of claim 1 wherein said vector is a VEE virus replicon vector.

6. The recombinant DNA construct according to claim 5 wherein said MBGV virus proteins are from strain Musoke.

7. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus GP.

8. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus NP.

9. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP40.

10. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP35.

11. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP30.

12. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP24.

13. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus GPΔTM.

14. Self replicating RNA produced from the construct of any of claim 7, 8, 9, 10, 11, 12, or 13.

15. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 14.

16. A pharmaceutical composition comprising infectious alphavirus particles according to claim 15 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

17. A host cell transformed with a recombinant DNA construct according to claim 5.

18. A host cell according to claim 17 wherein said host cell is prokaryotic.

19. A host cell according to claim 17 wherein said host cell is eukaryotic.

20. A method for producing MBGV virus proteins comprising culturing the cells according to claim 18 under conditions such that said DNA fragment is expressed and said MBGV protein is produced.

21. A method for producing MBGV proteins comprising culturing the cells according to claim 19 under conditions such that said DNA fragment is expressed and said MBGV protein is produced.

22. A vaccine for MBGV comprising viral particles containing one or more replicon RNA encoding one or more MBGV proteins selected from the group consisting of GP, NP, VP24, VP30, VP35, VP40, and GPΔTM.

23. A pharmaceutical composition comprising the self replication RNA of claim 14 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

24. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of pRep Mus GP, pRep Mus GPΔTM, pRep Mus NP, pRep Mus VP40, pRep Mus VP35, pRep Mus VP30, pRep Mus VP24 in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier/and or adjuvant.

L5 ANSWER 5 OF 6 USPATFULL on STN
2003:40417 **Marburg** virus vaccines.
Hevey, Michael C., Frederick, MD, United States
Negley, Diane L., Frederick, MD, United States
Pushko, Peter, Frederick, MD, United States
Smith, Jonathan F., Sabillasville, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6517842 B1 20030211
APPLICATION: US 1999-336910 19990621 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention here relates to recombinant DNA constructs which comprise a Venezuelan equine encephalitis replicon vector and at least one DNA fragment encoding a protective antigen from the **Marburg** virus. The DNA constructs are useful for inducing an immune response which is protective against infection with **Marburg** virus in nonhuman primates.
CLM What is claimed is:

1. A recombinant DNA construct comprising: (i) a Venezuelan equine encephalitis replicon vector, and (ii) at least one DNA fragment encoding a protective antigen from the Musoke strain of the **Marburg** virus.

2. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus GP.

3. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus NP.

4. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP40.

5. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP35.

6. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP30.

7. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP24.

8. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus GPΔTM.

9. A host cell transformed with a recombinant DNA construct according to claim 1.

10. A host cell according to claim 9 wherein said host cell is prokaryotic.

11. A host cell according to claim 9 wherein said host cell is eukaryotic.

12. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of pRep Mus GP, pRep Mus GPΔTM, pRep Mus NP, pRep Mus VP40, pRep Mus VP35, pRep Mus VP30, pRep Mus VP24 in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier/and or adjuvant.

L5 ANSWER 6 OF 6 USPATFULL on STN
2002:294539 **EBOLA** VIRION PROTEINS EXPRESSED FROM VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS REPLICONS.
HART, MARY K., FREDERICK, MD, UNITED STATES
WILSON, JULIE A., FREDERICK, MD, UNITED STATES
PUSHKO, PETER, FREDERICK, MD, UNITED STATES
SMITH, JONATHAN F., SABILLASVILLE, MD, UNITED STATES
SCHMALJOHN, ALAN L., FREDERICK, MD, UNITED STATES
US 2002164582 A1 20021107
APPLICATION: US 1999-337946 A1 19990622 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Using the **Ebola** GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is protective against infection with **Ebola** virus is described.

CLM What is claimed is:

1. A DNA fragment which encodes a GP **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:1, or a polynucleotide fragment comprising at least 15 nucleotides.

2. A DNA fragment which encodes a NP **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:2, or a polynucleotide fragment comprising at least 15 nucleotides.

3. A DNA fragment which encodes a VP24 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:3, or a polynucleotide fragment comprising at least 15 nucleotides.

4. A DNA fragment which encodes a VP30 **Ebola** protein, said DNA fragment comprising the sequence specified in any of SEQ ID NO:4 and SEQ ID NO:7, or a polynucleotide fragment comprising at least 15 nucleotides.

5. A DNA fragment which encodes a VP35 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:5, or a polynucleotide fragment comprising at least 15 nucleotides.

6. A DNA fragment which encodes a VP40 **Ebola** protein, said DNA fragment comprising the sequence specified in SEQ ID NO:6, or a polynucleotide fragment comprising at least 15 nucleotides.

7. A DNA fragment which encodes a GP **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:17 or a conservative substitution thereof.

8. A DNA fragment which encodes a NP **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:18 or a conservative substitution thereof.

9. A DNA fragment which encodes a VP24 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:19 or a conservative substitution thereof.

10. A DNA fragment which encodes a VP30 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in any of SEQ ID NO:20 and SEQ ID NO:23 or a conservative substitution thereof.

11. A DNA fragment which encodes a VP35 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:21 or a conservative substitution thereof.

12. A DNA fragment which encodes a VP40 **Ebola** protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:22 or a conservative substitution thereof.

13. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the **Ebola** virus DNA fragments chosen from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6 and 7 or a fragment thereof comprising at least 15 nucleotides.

14. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the **Ebola** virus DNA fragments chosen from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24 and 25 or a conservative substitution thereof.

15. The recombinant DNA construct of claim 13 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

16. The recombinant DNA construct of claim 14 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

17. A recombinant DNA construct according to claim 13 wherein said vector is an expression vector.

18. A recombinant DNA construct according to claim 13 wherein said vector is a prokaryotic vector.

19. A recombinant DNA construct according to claim 13 wherein said vector is a eukaryotic vector.

20. A recombinant DNA construct according to claim 14 wherein said vector is an expression vector.

21. A recombinant DNA construct according to claim 14 wherein said vector is a prokaryotic vector.

22. A recombinant DNA construct according to claim 14 wherein said vector is a eukaryotic vector.

23. The recombinant DNA construct of claim 17 wherein said vector is a VEE virus replicon vector.

24. The recombinant DNA construct of claim 20 wherein said vector is a VEE virus replicon vector.

25. The recombinant DNA construct according to claim 23 wherein said **Ebola** virus DNA fragments are from **Ebola** Zaire 1976.

26. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP24.

27. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP30.

28. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP35.

29. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP40.

30. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboNP.

31. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboGP.

32. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboVP30(#2).

33. Self replicating RNA produced from a construct chosen from the group consisting of EboVP24ReP, EboVP30ReP, EboVP35ReP, EboVP40ReP, EboVPNPReP, EboVPGPReP, and EboVP30ReP(#2).

34. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 33.

35. A pharmaceutical composition comprising infectious alphavirus particles according to claim 34 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

36. A host cell transformed with a recombinant DNA construct according to claim 13.

37. A host cell transformed with a recombinant DNA construct according to claim 14.

38. A host cell according to claim 36 wherein said host cell is prokaryotic.

39. A host cell according to claim 36 wherein said host cell is eukaryotic.

40. A host cell according to claim 37 wherein said host cell is prokaryotic.

41. A host cell according to claim 37 wherein said host cell is eukaryotic.

42. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 36 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

43. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 37 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

44. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 38 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

45. A method for producing **Ebola** virus proteins comprising culturing the cells according to claim 39 under conditions such that said DNA fragment is expressed and said **Ebola** protein is produced.

46. An isolated and purified **Ebola** GP protein specified in SEQ ID NO:17 and conservative substitutions thereof, or an immunologically identifiable portion thereof.

47. An isolated and purified **Ebola** NP protein specified in SEQ ID NO:18 and conservative substitutions thereof or an immunologically identifiable portion thereof.

48. An isolated and purified **Ebola** VP24 protein specified in SEQ ID NO:19 and conservative substitutions thereof or an immunologically identifiable portion thereof.

49. An isolated and purified **Ebola** VP30 protein specified in any of SEQ ID NO:20 and SEQ ID NO:23 and conservative substitutions thereof or an immunologically identifiable portion thereof.

50. An isolated and purified **Ebola** VP35 protein specified in SEQ ID NO:21 and conservative substitutions thereof or an immunologically identifiable portion thereof.

51. An isolated and purified **Ebola** VP40 protein specified in SEQ ID NO:22 and conservative substitutions thereof or an immunologically identifiable portion thereof.

52. An antibody to a peptide encoded by the sequence specified in SEQ ID NO:17, 18, 19, 20, 21, 22, 23, 24, and 25.

53. A method for detecting **Ebola** virus infection comprising contacting a sample from a subject suspected of having **Ebola** virus infection with a antibody according to claim 52 and detecting the presence or absence by detecting the presence or absence of a complex formed between the **Ebola** protein and antibodies specific therefor.

54. A method for detecting the presence or absence of **Ebola** virus GP RNA in a sample using the polymerase chain reaction using primers for **Ebola** GP nucleic acid sequence specified in SEQ ID NO:1 for GP.

55. An **Ebola** infection diagnostic kit comprising at least 12 consecutive nucleotides of SEQ ID NO:1 specific for the amplification of DNA or RNA of **Ebola** virus in a sample using the polymerase chain reaction and ancillary reagents suitable for use in such a reaction for detecting the presence or absence of **Ebola** virus DNA or RNA in a sample.

56. A vaccine for **Ebola** comprising alphavirus particles of claim 34.

57. A method for the diagnosis of **Ebola** virus infection comprising the steps of: (i) contacting a sample from an individual suspected of having **Ebola** virus infection with an antibody to **Ebola** proteins according to claim 52; and (ii) detecting the presence or absence of **Ebola** virus infection by detecting the presence or absence of a complex formed between **Ebola** proteins and antibodies specific therefor.

58. A pharmaceutical composition comprising the self replicating RNA of claim 33 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

59. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of VRepEboVP24, VRepEboVP30, VRepEboVP35, VRepEboVP40, VRepEboNP, VRepEboGP, and VRepEboVP30(#2), in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier and/or adjuvant.

60. A pharmaceutical composition comprising comprising a peptide encoded by any of SEQ ID NO:24 and SEQ ID NO:25, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

```
=> d his

     (FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005)

     FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
L1          2139 S (FILOVIR? OR MARBURG OR EBOLA)
L2            35 S L1 AND (GP1 OR GP2)
L3           255 S L1 AND (BIVALENT OR MULTIVALENT)
L4             8 S L3 AND (MARBURG/TI OR EBOLA/TI OR FILOVIR?/TI)
L5             6 S L4 NOT L2

=> d l5,cbib,kwic,5

L5   ANSWER 5 OF 6  USPATFULL on STN
2003:40417 Marburg virus vaccines.
    Hevey, Michael C., Frederick, MD, United States
    Negley, Diane L., Frederick, MD, United States
    Pushko, Peter, Frederick, MD, United States
    Smith, Jonathan F., Sabillasville, MD, United States
    Schmaljohn, Alan L., Frederick, MD, United States
    The United States of America as represented by the Secretary of the Army,
    Washington, DC, United States (U.S. government)
    US 6517842 B1 20030211
    APPLICATION: US 1999-336910  19990621 (9)
    PRIORITY: US 1998-91403P  19980629 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI     Marburg virus vaccines
AB     . . . which comprise a Venezuelan equine encephalitis replicon vector
```

and at least one DNA fragment encoding a protective antigen from the **Marburg** virus. The DNA constructs are useful for inducing an immune response which is protective against infection with **Marburg** virus in nonhuman primates.

SUMM **Marburg** virus (MBGV) was first recognized in 1967, when an outbreak of hemorrhagic fever in humans occurred in Germany and Yugoslavia,. . . filamentous morphology of the virus was later recognized to be characteristic, not only of additional MBGV isolates, but also of **Ebola** virus (EBOV) (Johnson et al., 1977, J. Virol. 71, 3031-3038; Smith et al., 1982, Lancet 1, 816-820; Pattyn et al., 1977, Lancet 1, 573-574). MBGV and EBOV are now known to be distinctly different lineages in the family **Filoviridae**, within the viral order Mononegavirales (Kiley et al., 1982, Intervirology 18, 24-32; Feldmann and Klenk, 1996, Adv. Virus Res. 47,. . .

SUMM Irrespective of how encouraging **filovirus** vaccine results may appear in guinea pigs or mice, protection of nonhuman primates is widely taken as the more definitive. . . in guinea pigs or mice. Small animal models with fatal disease outcomes have been achieved only with a subset of **filovirus** isolates and only then by multiple serial passages in the desired host (Hevey et al., 1997, supra; Connolly et al.,. . .

SUMM Therefore, there is a need for an efficacious vaccine for MBGV useful for protecting humans against **Marburg** hemorrhagic fever.

DETD **Filoviruses**. The **filoviruses** (e.g. **Marburg** virus, MBGV) cause acute hemorrhagic fever characterized by high mortality. Humans can contract **filoviruses** by infection in endemic regions, by contact with imported primates, and by performing scientific research with the virus. However, there currently are no available vaccines or effective therapeutic treatments for **filovirus** infection. The virions of **filoviruses** contain seven proteins which include a surface glycoprotein (GP), a nucleoprotein (NP), an RNA-dependent RNA polymerase (L), and four virion. . .

DETD . . . a DNA sequence encoding any of MBGV virion proteins GP, GPΔTM, NP, VP40, VP35, VP30, VP24. The sequences encoding the **Marburg** proteins GP, GPΔTM, NP, VP40, VP35, VP30, VP24 corresponding to nucleotides 104-11242 of the Genbank sequence is presented in SEQ. . .

DETD . . . below in Materials and Methods. Schematic diagrams of the resulting constructs are shown in the Figures. The VEE constructs containing **Marburg** proteins can be used as a DNA vaccine, or for the production of RNA molecules as described below.

DETD . . . a replicon particle, resulting from one of the replicon constructs described above, or a combination of replicon particles as a **multivalent** vaccine, in combination with a pharmaceutically acceptable carrier. An "immunogenic amount" is an amount of the replicon particles sufficient to. . .

DETD

TABLE 1

Protection of replicon inoculated strain 13 guinea pigs from lethal challenge with **Marburg** virus (Musoke isolate)

Log 10 ELISA Titer* Anti-gen	Replicon	# of Doses S/T[a]	Day-7	Day 64 Viremia[b]	# of Doses V/T[c]	MDD
GP 3. . .						

DETD . . . observed, at 1:20 or higher dilutions, in any sample. It should be noted that it is frequently difficult to demonstrate **filovirus** neutralizing antibody in vitro; however, antibodies may nonetheless be relevant in vivo (Hevey et al., 1997, Virology 239, 206-216), perhaps. . .

DETD To our knowledge, this is the first report of any **filovirus** vaccine shown to be completely efficacious in nonhuman primates. Before these observations, we were cautiously optimistic about the overall feasibility of an efficacious vaccine for MBGV, but were also concerned that proofs of **filovirus** vaccine concepts in guinea pigs may not necessarily forecast success in nonhuman primates and, by inference, in humans. Results presented. . .

DETD

GENERAL INFORMATION:

NUMBER OF SEQ ID NOs: 7

SEQUENCE CHARACTERISTICS:

SEQ ID NO: 1

LENGTH: 11460

TYPE: DNA

ORGANISM: **Marburg** Virus

SEQUENCE: 1

agacacacaa aaacaagaga tgatgatttt gtgtatcata 40

taaataaaga agaatattaa cattgacatt gagacttgtc 80

agtcgtgtaa tattcttgaa gatatggatt tacacagttt 120

gttggagttg ggtacaaaac ccactgcccc tcatgtccgt 160
aataagaaag tgatattatt tgacacaaat. . . tttcacaggc tgctaaactt actaaatttt
11360
acataggatt atataattct tttcgataca cgttatatct 11400
ttagcaaagt gaggaaaaca gctttatcat gttagatgcc 11440
agttatccat tttaagtgaa 11460
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 681
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 2
Met Lys Thr Thr Cys Phe Leu Ile Ser Leu Ile Leu Ile Gln Gly
1 5 10 15
Thr. . .
DETD . . . Cys Ile Cys Arg Ile
665 670 675
Phe Thr Lys Tyr Ile Gly
680
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 693
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 3
Met Asp Leu His Ser Leu Leu Glu Leu Gly Thr Pro Thr Ala Pro
1 5 10 15
His. . . Pro Asp Met Ser Phe Asp Glu Gly Asp
680 685 690
Met Leu Arg
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4
LENGTH: 303
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 4
Met Ala Ser Ser Ser Asn Tyr Asn Thr Tyr Met Gln Tyr Leu Asn
1 5 10 15
Ser. . . Val Leu Ala Tyr Ala Asn Pro Thr Leu
290 295 300
Ser Ala Val
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 329
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 5
Met Trp Asp Ser Ser Tyr Met Gln Gln Val Ser Glu Gly Leu Met
1 5 10 15
Thr. . . Ser Ser Glu Gln Gly Glu Thr Arg Ala Leu Lys Ile
320 325
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 277
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 6
Met Gln Gln Pro Arg Gly Arg Ser Arg Thr Arg Asn His Gln Val
1 5 10 15
Thr. . . Tyr Glu Ser Phe
260 265 270
Tyr Ser Ser Ser Lys Ser Arg
275
SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7
LENGTH: 253
TYPE: PRT
ORGANISM: **Marburg** Virus
SEQUENCE: 7
Met Ala Glu Leu Ser Thr Arg Tyr Asn Leu Pro Ala Asn Val Thr
1 5 10 15
Glu. . .
CLM What is claimed is:
. . . encephalitis replicon vector, and (ii) at least one DNA fragment encoding a protective antigen from the Musoke strain of the **Marburg** virus.

```
=> d his

     (FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005)

     FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
L1          2139 S (FILOVIR? OR MARBURG OR EBOLA)
L2            35 S L1 AND (GP1 OR GP2)
L3           255 S L1 AND (BIVALENT OR MULTIVALENT)
L4             8 S L3 AND (MARBURG/TI OR EBOLA/TI OR FILOVIR?/TI)
L5             6 S L4 NOT L2

=> e grogan case c/in
E1             1     GROFT WILLIAM E/IN
E2             1     GROFTE THORBJORN/IN
E3             1 --> GROGAN CASE C/IN
E4             1     GROGAN CATHERINE/IN
E5             3     GROGAN CHARLES W/IN
E6             4     GROGAN DANIEL/IN
E7             1     GROGAN DANIEL R/IN
E8             1     GROGAN DANIEL W/IN
E9             1     GROGAN DARREN/IN
E10            2     GROGAN DENISE C/IN
E11            1     GROGAN DENNIS/IN
E12            1     GROGAN DENNIS R/IN

=> s e3
L6             1 "GROGAN CASE C"/IN

=> d l6,cbib

L6   ANSWER 1 OF 1  USPATFULL on STN
2003:158947 Chimeric filovirus glycoprotein.
    Grogan, Case C., Gaithersburg, MD, UNITED STATES
    Hevey, Michael C., Frederick, MD, UNITED STATES
    Schmaljohn, Alan L., Frederick, MD, UNITED STATES
    US 2003108560 A1 20030612
    APPLICATION: US 2002-66506  A1  20020131 (10)
    PRIORITY: US 2001-267522P  20010131 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> e schmaljohn alan l/in
E1            26     SCHMALIX CHARLES KENNETH/IN
E2             1     SCHMALJOHN ALAN/IN
E3            10 --> SCHMALJOHN ALAN L/IN
E4            12     SCHMALJOHN CONNIE S/IN
E5             1     SCHMALL DAVID/IN
E6             2     SCHMALL KARL H/IN
E7            19     SCHMALL KARL HEINZ/IN
E8             1     SCHMALL MARKUS/IN
E9             3     SCHMALL ROBERT A/IN
E10            2     SCHMALLEGGER HELMUT/IN
E11            1     SCHMALLEGGER HERMANN/IN
E12            5     SCHMALLEGGER PETER/IN

=> s e2 or e3
               1 "SCHMALJOHN ALAN"/IN
              10 "SCHMALJOHN ALAN L"/IN
L7            11 "SCHMALJOHN ALAN"/IN OR "SCHMALJOHN ALAN L"/IN

=> s l7 not l6
L8            10 L7 NOT L6

=> d l8,cbib,1-10

L8   ANSWER 1 OF 10  USPATFULL on STN
2004:190090 Ebola virion proteins expressed from venezuelan equine encephalitis
    (VEE) virus replicons.
    Hart, Mary K., Frederick, MD, UNITED STATES
    Wilson, Julie A., Frederick, MD, UNITED STATES
    Pushko, Peter, Frederick, MD, UNITED STATES
    Smith, Jonathan F., Sabillasville, MD, UNITED STATES
    Schmaljohn, Alan L., Frederick, MD, UNITED STATES
    US 2004146859 A1 20040729
    APPLICATION: US 2003-696633  A1  20031029 (10)
    PRIORITY: US 1998-91403P  19980629 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

L8 ANSWER 2 OF 10 USPATFULL on STN
2004:76190 Generation of virus-like particles and demonstration of lipid rafts as sites of filovirus entry and budding.
Bavari, Sina, Frederick, MD, UNITED STATES
Aman, M. Javad, Potomac, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2004057967 A1 20040325
APPLICATION: US 2002-289839 A1 20021107 (10)
PRIORITY: US 2001-338936P 20011107 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 10 USPATFULL on STN
2003:268051 Monoclonal antibodies to Ebola glycoprotein.
Hart, Mary K., Frederick, MD, United States
Wilson, Julie A., Frederick, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6630144 B1 20031007
APPLICATION: US 2000-650086 20000829 (9)
PRIORITY: US 1999-151505P 19990830 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 4 OF 10 USPATFULL on STN
2003:219300 Marburg virus vaccines.
Hevey, Michael C., Frederick, MD, UNITED STATES
Negley, Diane L., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2003152590 A1 20030814
APPLICATION: US 2002-267322 A1 20021009 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 5 OF 10 USPATFULL on STN
2003:40417 Marburg virus vaccines.
Hevey, Michael C., Frederick, MD, United States
Negley, Diane L., Frederick, MD, United States
Pushko, Peter, Frederick, MD, United States
Smith, Jonathan F., Sabillasville, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6517842 B1 20030211
APPLICATION: US 1999-336910 19990621 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 6 OF 10 USPATFULL on STN
2003:30279 Prophylactic and therapeutic monoclonal antibodies.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
Schmaljohn, Connie S., Frederick, MD, UNITED STATES
US 2003022226 A1 20030130
APPLICATION: US 2002-202532 A1 20020916 (10)
PRIORITY: US 2000-182066P 20000211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 7 OF 10 USPATFULL on STN
2002:314399 DNA vaccines against poxviruses.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
Schmaljohn, Connie S., Frederick, MD, UNITED STATES
US 2002176871 A1 20021128
APPLICATION: US 2001-800632 A1 20010307 (9)
PRIORITY: US 2000-187608P 20000307 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 8 OF 10 USPATFULL on STN
2002:294539 EBOLA VIRION PROTEINS EXPRESSED FROM VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS REPLICONS.

HART, MARY K., FREDERICK, MD, UNITED STATES
WILSON, JULIE A., FREDERICK, MD, UNITED STATES
PUSHKO, PETER, FREDERICK, MD, UNITED STATES
SMITH, JONATHAN F., SABILLASVILLE, MD, UNITED STATES
SCHMALJOHN, ALAN L., FREDERICK, MD, UNITED STATES
US 2002164582 A1 20021107
APPLICATION: US 1999-337946 A1 19990622 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 9 OF 10 USPATFULL on STN
2002:16571 Prophylactic and therapeutic monoclonal antibodies.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
Schmaljohn, Connie S., Frederick, MD, UNITED STATES
US 2002009447 A1 20020124
APPLICATION: US 2001-781124 A1 20010209 (9)
PRIORITY: US 2000-182066P 20000211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 10 OF 10 USPATFULL on STN
2001:36806 Genetic induction of anti-viral immune response and genetic vaccine for filovirus.
Haynes, Joel R., Fort Collins, CO, United States
Schmaljohn, Connie S., Frederick, MD, United States
Fuller, Deborah L., Oregon, WI, United States
Schmaljohn, Alan, Frederick, MD, United States
Jahrling, Peter B., Middletown, MD, United States
PowerJect Vaccines Inc., Madison, WI, United States (U.S. corporation)
US 6200959 B1 20010313
APPLICATION: US 1996-760615 19961204 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l8,cbib,ab,clm,1-10

L8 ANSWER 1 OF 10 USPATFULL on STN
2004:190090 Ebola virion proteins expressed from venezuelan equine encephalitis (VEE) virus replicons.
Hart, Mary K., Frederick, MD, UNITED STATES
Wilson, Julie A., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2004146859 A1 20040729
APPLICATION: US 2003-696633 A1 20031029 (10)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Using the Ebola GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is protective against infection with Ebola virus is described.

CLM What is claimed is:

1. A DNA fragment which encodes a GP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:1, or a polynucleotide fragment comprising at least 15 nucleotides.

2. A DNA fragment which encodes a NP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:2, or a polynucleotide fragment comprising at least 15 nucleotides.

3. A DNA fragment which encodes a VP24 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:3, or a polynucleotide fragment comprising at least 15 nucleotides.

4. A DNA fragment which encodes a VP30 Ebola protein, said DNA fragment comprising the sequence specified in any of SEQ ID NO:4 and SEQ ID NO:7, or a polynucleotide fragment comprising at least 15 nucleotides.

5. A DNA fragment which encodes a VP35 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:5, or a polynucleotide fragment comprising at least 15 nucleotides.

6. A DNA fragment which encodes a VP40 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:6, or a polynucleotide fragment comprising at least 15 nucleotides.

7. A DNA fragment which encodes a GP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:17 or a conservative substitution thereof.

8. A DNA fragment which encodes a NP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:18 or a conservative substitution thereof.

9. A DNA fragment which encodes a VP24 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:19 or a conservative substitution thereof.

10. A DNA fragment which encodes a VP30 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in any of SEQ ID NO:20 and SEQ ID NO:23 or a conservative substitution thereof.

11. A DNA fragment which encodes a VP35 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:21 or a conservative substitution thereof.

12. A DNA fragment which encodes a VP40 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:22 or a conservative substitution thereof.

13. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6 and 7 or a fragment thereof comprising at least 15 nucleotides.

14. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24 and 25 or a conservative substitution thereof.

15. The recombinant DNA construct of claim 13 wherein said DNA fragment induces a cytotoxic T lymphocyte, response or antibody response.

16. The recombinant DNA construct of claim 14 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

17. A recombinant DNA construct according to claim 13 wherein said vector is an expression vector.

18. A recombinant DNA construct according to claim 13 wherein said vector is a prokaryotic vector.

19. A recombinant DNA construct according to claim 13 wherein said vector is a eukaryotic vector.

20. A recombinant DNA construct according to claim 14 wherein said vector is an expression vector.

21. A recombinant DNA construct according to claim 14 wherein said vector is a prokaryotic vector.

22. A recombinant DNA construct according to claim 14 wherein said vector is a eukaryotic vector.

23. The recombinant DNA construct of claim 17 wherein said vector is a VEE virus replicon vector.

24. The recombinant DNA construct of claim 20 wherein said vector is a VEE virus replicon vector.

25. The recombinant DNA construct according to claim 23 wherein said Ebola virus DNA fragments are from Ebola Zaire 1976.

26. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP24.

27. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP30.

28. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP35.

29. The recombinant DNA construct according to claim 25 wherein said

construct is VRepEboVP40.

30. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboNP.

31. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboGP.

32. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboVP30(#2).

33. Self replicating RNA produced from a construct chosen from the group consisting of EboVP24ReP, EboVP30ReP, EboVP35ReP, EboVP40ReP, EboVPNPReP, EboVPGPReP, and EboVP30ReP(#2).

34. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 33.

35. A pharmaceutical composition comprising infectious alphavirus particles according to claim 34 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

36. A host cell transformed with a recombinant DNA construct according to claim 13.

37. A host cell transformed with a recombinant DNA construct according to claim 14.

38. A host cell according to claim 36 wherein said host cell is prokaryotic.

39. A host cell according to claim 36 wherein said host cell is eukaryotic.

40. A host cell according to claim 37 wherein said host cell is prokaryotic.

41. A host cell according to claim 37 wherein said host cell is eukaryotic.

42. A method for producing Ebola virus proteins comprising culturing the cells according to claim 36 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

43. A method for producing Ebola virus proteins comprising culturing the cells according to claim 37 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

44. A method for producing Ebola virus proteins comprising culturing the cells according to claim 38 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

45. A method for producing Ebola virus proteins comprising culturing the cells according to claim 39 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

46. An isolated and purified Ebola GP protein specified in SEQ ID NO:17 and conservative substitutions thereof, or an immunologically identifiable portion thereof.

47. An isolated and purified Ebola NP protein specified in SEQ ID NO:18 and conservative substitutions thereof or an immunologically identifiable portion thereof.

48. An isolated and purified Ebola VP24 protein specified in SEQ ID NO:19 and conservative substitutions thereof or an immunologically identifiable portion thereof.

49. An isolated and purified Ebola VP30 protein specified in any of SEQ ID NO:20 and SEQ ID NO:23 and conservative substitutions thereof or an immunologically identifiable portion thereof.

50. An isolated and purified Ebola VP35 protein specified in SEQ ID NO:21 and conservative substitutions thereof or an immunologically identifiable portion thereof.

51. An isolated and purified Ebola VP40 protein specified in SEQ ID NO:22 and conservative substitutions thereof or an immunologically identifiable portion thereof.

52. An antibody to a peptide encoded by the sequence specified in SEQ ID NO:17, 18, 19, 20, 21, 22, 23, 24, and 25.

53. A method for detecting Ebola virus infection comprising contacting a sample from a subject suspected of having Ebola virus infection with a antibody according to claim 52 and detecting the presence or absence by detecting the presence or absence of a complex formed between the Ebola protein and antibodies specific therefor.

54. A method for detecting the presence or absence of Ebola virus GP RNA in a sample using the polymerase chain reaction using primers for Ebola GP nucleic acid sequence specified in SEQ ID NO:1 for GP.

55. An Ebola infection diagnostic kit comprising at least 12 consecutive nucleotides of SEQ ID NO:1 specific for the amplification of DNA or RNA of Ebola virus in a sample using the polymerase chain reaction and ancillary reagents suitable for use in such a reaction for detecting the presence or absence of Ebola virus DNA or RNA in a sample.

56. A vaccine for Ebola comprising alphavirus particles of claim 34.

57. A method for the diagnosis of Ebola virus infection comprising the steps of: (i) contacting a sample from an individual suspected of having Ebola virus infection with an antibody to Ebola proteins according to claim 52; and (ii) detecting the presence or absence of Ebola virus infection by detecting the presence or absence of a complex formed between Ebola proteins and antibodies specific therefor.

58. A pharmaceutical composition comprising the self replicating RNA of claim 33 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

59. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of VRepEboVP24, VRepEboVP30, VRepEboVP35, VRepEboVP40, VRepEboNP, VRepEboGP, and VRepEboVP30(#2), in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier and/or adjuvant.

60. A pharmaceutical composition comprising comprising a peptide encoded by any of SEQ ID NO:24 and SEQ ID NO:25, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

L8 ANSWER 2 OF 10 USPATFULL on STN
2004:76190 Generation of virus-like particles and demonstration of lipid rafts as sites of filovirus entry and budding.
Bavari, Sina, Frederick, MD, UNITED STATES
Aman, M. Javad, Potomac, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2004057967 A1 20040325
APPLICATION: US 2002-289839 A1 20021107 (10)
PRIORITY: US 2001-338936P 20011107 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB In this application is described a method for the formation of filovirus-like particles for both Ebola and Marburg and their use as a diagnostic and therapeutic agent as well as a filovirus vaccine. Also described is the association of Ebola and Marburg with lipid rafts during assembly and budding, and the requirement of functional rafts for entry of filoviruses into cells.
CLM What is claimed is:
1. A filovirus virus like particle, VLP, comprising filovirus envelope glycoprotein, GP, and filovirus matrix protein, VP40.

2. A filovirus VLP, produced by expressing in a cell a polynucleotide encoding filovirus envelope glycoprotein, GP, and filovirus matrix protein, VP40 such that said polynucleotide is expressed and said VLP is produced.

3. A VLP of claim 1 where said filovirus is chosen from the group consisting of Ebola and Marburg.

4. A VLP of claim 2 where said filovirus is chosen from the group consisting of Ebola and Marburg.

5. A method for inhibiting the association of a filovirus envelope glycoprotein GP with lipid rafts, comprising inhibiting palmitoylation

at cysteine residues 670 and 672 of said GP.

6. A method for preventing filovirus trafficking into and out of a cell comprising disrupting lipid rafts of said cell.

7. The method of claim 5 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

8. The method of claim 6 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

9. The method according to claim 6 wherein said rafts are disrupted with a cholesterol destabilizing agent.

10. The method according to claim 8 wherein said agents are filipin and nystatin.

11. A method for preventing filovirus trafficking said method comprising introducing to a cell cholesterol synthesis inhibitors.

12. The method of claim 11 wherein said cholesterol synthesis inhibitor is methyl-β-cyclodextrin.

13. A filovirus vaccine comprising VLP according to claim 1.

14. A filovirus vaccine comprising VLP according to claim 2.

15. A filovirus vaccine according to claim 13 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

16. A filovirus vaccine according to claim 14 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

17. A filovirus vaccine comprising VLP and a nucleic acid encoding an agent capable of eliciting an immune response against said filovirus.

18. A method for introducing an agent into a cell, comprising packaging said agent into a VLP producing a packed VLP and allowing the packed VLP to enter said cell.

19. The method according to claim 18 wherein said VLP is that of claim 1.

20. The method according to claim 19 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

21. The method according to claim 18 wherein said VLP is that of claim 2.

22. The method according to claim 21 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

23. A method for testing an agent involved in filovirus budding, comprising introducing said agent to a cultured cell producing filovirus VLP and monitoring the presence or absence of a change in the budding of VLP as compared to a control by measuring VLPs in supernatant of said cultured cell, wherein a reduction or increase in the number of VLP in the superntant indicates a negative or positive agent, respectively, on filovirus budding.

24. The method according to claim 23 wherein said filovirus is chosen from the group consisting of Ebola and Marburg.

25. A method for inhibiting Ebola virus infection in a cell comprising administering to said cell lipid raft-disrupting agents.

26. The method according to claim 25 wherein said agents are Filipin and Nystatin.

27. A method for detecting Ebola virus infection comprising contacting a sample from a subject suspected of having Ebola virus infection with an Ebola VLP according to claim 3 and detecting the presence or absence of an infection by detecting the presence or absence of a complex formed between the Ebola VLP and antibodies specific therefor in said sample.

28. A kit for the detection of Ebola virus infection comprising Ebola VLPs according to claim 3.

29. A method for detecting Marburg virus infection comprising contacting

a sample from a subject suspected of having Marburg virus infection with a Marburg VLP according to claim 3 and detecting the presence or absence of an infection by detecting the presence or absence of a complex formed between the Marburg VLP and antibodies specific therefor in said sample.

30. A kit for the detection of Marburg virus infection comprising Marburg VLPs according to claim 3.

31. A kit for testing agents involved in Ebola budding said kit comprising a cell producing Ebola VLPs and ancillary reagents for detecting VLPs in the supernatant of said cells when cells are cultured.

32. An Ebola VLP-producing cell comprising a mammalian cell expressing Ebola GP and VP40.

33. A kit for testing agents involved in Marburg budding said kit comprising a cell producing Marburg VLPs and ancillary reagents for detecting VLPs in the supernatant of said cells when cells are cultured.

34. A Marburg VLP-producing cell comprising a mammalian cell expressing Marburg GP and VP40.

35. An immunogenic composition comprising, in a physiologically acceptable vehicle, Ebola VLPs.

36. The immunogenic composition according to claim 35, which induces an Ebola specific immune response in a subject.

37. The immunogenic composition according to claim 35 which further comprises an adjuvant to enhance the immune response.

38. The immunogenic composition of claim 35, wherein said Ebola VLPs are produced by expressing in a mammalian cell Ebola GP and Ebola VP40.

39. A method for stimulating an Ebola virus specific immune response, said method comprising administering to a subject an immunologically sufficient amount of Ebola VLPs in a physiologically acceptable vehicle.

40. An immunogenic composition comprising, in a physiologically acceptable vehicle, Marburg VLPs.

41. The immunogenic composition according to claim 40, which induces a Marburg specific immune response in a subject.

42. The immunogenic composition according to claim 40 which further comprises an adjuvant to enhance the immune response.

43. The immunogenic composition of claim 40, wherein said Marburg VLPs are produced by expressing in a mammalian cell Marburg GP and Marburg VP40.

44. A method for stimulating a Marburg virus specific immune response, said method comprising administering to a subject an immunologically sufficient amount of Marburg VLPs in a physiologically acceptable vehicle.

L8 ANSWER 3 OF 10 USPATFULL on STN
2003:268051 Monoclonal antibodies to Ebola glycoprotein.
Hart, Mary K., Frederick, MD, United States
Wilson, Julie A., Frederick, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6630144 B1 20031007
APPLICATION: US 2000-650086 20000829 (9)
PRIORITY: US 1999-151505P 19990830 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB In this application are described Ebola GP monoclonal antibodies and epitopes recognized by these monoclonal antibodies. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention, and/or therapeutical treatment of Ebola virus infections in vitro and in vivo.
CLM What is claimed:
1. An isolated monoclonal antibody which recognizes Ebola virus GP, wherein the epitope that binds or is recognized by said antibody is within SEQ ID NO:6 or SEQ ID NO:8.

2. The antibody according to claim 1, wherein the antibody binds Ebola virus in vitro.

3. The antibody according to claim 1, wherein the antibody immunoprecipitates GP from supernatants or cell lysates of cell cultures infected with Ebola virus.

4. The antibody according to claim 1 wherein said epitope is within SEQ ID NO:6, and is further within SEQ ID NO:7.

5. The antibody according to claim 1 wherein said epitope is within SEQ ID NO:8, and is further within SEQ ID NO:9.

6. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 13F6-1-2 with Accession no. PTA-373.

7. An antibody which competes with the antibody of claim 6 for binding to Ebola virus GP.

8. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 6D3-1-1 with Accession no. PTA-374.

9. An antibody which competes with the antibody of claim 8 for binding to Ebola virus GP.

10. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 13C6-1-1 with Accession no. PTA-375.

11. An antibody which competes with the antibody of claim 10 for binding to Ebola virus GP.

12. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 6D8-1-2 with Accession no. PTA-376.

13. An antibody which competes with the antibody of claim 12 for binding to Ebola virus GP.

14. The antibody according to claim 1, wherein said antibody is produced by hybridoma cell line EGP 12B5-1-1 with Accession no. PTA-436.

15. An antibody which competes with the antibody of claim 14 for binding to Ebola virus GP.

16. A mixture comprising Ebola virus antibodies comprising one or more antibodies selected from the group consisting of an antibody produced by hybridoma EGP 13F6-1-2 accession no. PTA 373; an antibody produced by hybridoma EGP 6D3-1-1 accession no. PTA 374; an antibody produced by hybridoma EGP 13C6-1-1 accession no. PTA 375; an antibody produced by hybridoma EGP 6D8-1-2 accession no. PTA 376; and an antibody produced by hybridoma EGP 12B5-1-1 accession no. PTA 436.

17. A monoclonal antibody producing cell line that produces a monoclonal antibody according to claim 1.

18. The cell line according to claim 17, selected from the group consisting of cell line EGP 13F6-1-2 (ATCC accession no. PTA 373), cell line EGP 6D3-1-1 (ATCC accession no. PTA 374), cell line EGP 13C6-1-1 (ATCC accession no. PTA 375), cell line EGP 6D8-1-2 (ATCC accession no. PTA 376), and cell line EGP 12B5-1-1 (ATCC accession no. PTA 436).

19. An antiidiotypic antibody produced from any of the monoclonal antibodies selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

20. An antiidiotypic antibody produced from an antibody which competes for binding to GP with an antibody selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

L8 ANSWER 4 OF 10 USPATFULL on STN
2003:219300 Marburg virus vaccines.
Hevey, Michael C., Frederick, MD, UNITED STATES
Negley, Diane L., Frederick, MD, UNITED STATES
Pushko, Peter, Frederick, MD, UNITED STATES
Smith, Jonathan F., Sabillasville, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
US 2003152590 A1 20030814
APPLICATION: US 2002-267322 A1 20021009 (10)

PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Using the MBGV GP, NP, and virion proteins, a method and composition for use in inducing an immune response which is protective against infection with MBGV in nonhuman primates is described.
CLM What is claimed is:

1. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the MBGV virus DNA fragments encoding any one of GP, NP, VP40, VP35, VP30, VP24, and GPΔTM.

2. A recombinant DNA construct according to claim 1 wherein said vector is an expression vector.

3. A recombinant DNA construct according to claim 1 wherein said vector is a prokaryotic vector.

4. A recombinant DNA construct according to claim 1 wherein said vector is a eukaryotic vector.

5. The recombinant DNA construct of claim 1 wherein said vector is a VEE virus replicon vector.

6. The recombinant DNA construct according to claim 5 wherein said MBGV virus proteins are from strain Musoke.

7. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus GP.

8. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus NP.

9. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP40.

10. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP35.

11. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP30.

12. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus VP24.

13. The recombinant DNA construct according to claim 5 wherein said construct is pRep Mus GPΔTM.

14. Self replicating RNA produced from the construct of any of claim 7, 8, 9, 10, 11, 12, or 13.

15. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 14.

16. A pharmaceutical composition comprising infectious alphavirus particles according to claim 15 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

17. A host cell transformed with a recombinant DNA construct according to claim 5.

18. A host cell according to claim 17 wherein said host cell is prokaryotic.

19. A host cell according to claim 17 wherein said host cell is eukaryotic.

20. A method for producing MBGV virus proteins comprising culturing the cells according to claim 18 under conditions such that said DNA fragment is expressed and said MBGV protein is produced.

21. A method for producing MBGV proteins comprising culturing the cells according to claim 19 under conditions such that said DNA fragment is expressed and said MBGV protein is produced.

22. A vaccine for MBGV comprising viral particles containing one or more replicon RNA encoding one or more MBGV proteins selected from the group consisting of GP, NP, VP24, VP30, VP35, VP40, and GPΔTM.

23. A pharmaceutical composition comprising the self replication RNA of claim 14 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

24. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of pRep Mus GP, pRep Mus GPΔTM, pRep Mus NP, pRep Mus VP40, pRep Mus VP35, pRep Mus VP30, pRep Mus VP24 in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier/and or adjuvant.

L8 ANSWER 5 OF 10 USPATFULL on STN
2003:40417 Marburg virus vaccines.
Hevey, Michael C., Frederick, MD, United States
Negley, Diane L., Frederick, MD, United States
Pushko, Peter, Frederick, MD, United States
Smith, Jonathan F., Sabillasville, MD, United States
Schmaljohn, Alan L., Frederick, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6517842 B1 20030211
APPLICATION: US 1999-336910 19990621 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention here relates to recombinant DNA constructs which comprise a Venezuelan equine encephalitis replicon vector and at least one DNA fragment encoding a protective antigen from the Marburg virus. The DNA constructs are useful for inducing an immune response which is protective against infection with Marburg virus in nonhuman primates.
CLM What is claimed is:
1. A recombinant DNA construct comprising: (i) a Venezuelan equine encephalitis replicon vector, and (ii) at least one DNA fragment encoding a protective antigen from the Musoke strain of the Marburg virus.

2. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus GP.

3. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus NP.

4. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP40.

5. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP35.

6. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP30.

7. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus VP24.

8. The recombinant DNA construct according to claim 1 wherein said construct is pRep Mus GPΔTM.

9. A host cell transformed with a recombinant DNA construct according to claim 1.

10. A host cell according to claim 9 wherein said host cell is prokaryotic.

11. A host cell according to claim 9 wherein said host cell is eukaryotic.

12. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of pRep Mus GP, pRep Mus GPΔTM, pRep Mus NP, pRep Mus VP40, pRep Mus VP35, pRep Mus VP30, pRep Mus VP24 in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier/and or adjuvant.

L8 ANSWER 6 OF 10 USPATFULL on STN
2003:30279 Prophylactic and therapeutic monoclonal antibodies.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
Schmaljohn, Connie S., Frederick, MD, UNITED STATES

US 2003022226 A1 20030130
APPLICATION: US 2002-202532 A1 20020916 (10)
PRIORITY: US 2000-182066P 20000211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In this application are described vaccinia monoclonal antibodies. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention, and/or therapeutical treatment of vaccinia virus infections in vitro and in vivo.

CLM What is claimed is:

1. A composition comprising one or more monoclonal antibody directed against a vaccinia virus antigen.

2. The composition of claim 1 wherein said vaccinia virus antigen is L1R.

3. The composition of claim 1 wherein said vaccinia virus antigen is A33R.

4. The composition of claim 2 wherein said composition further comprises one or more monoclonal antibody directed against vaccinia A33R.

5. The composition of claim 4 wherein said composition further comprises one or more monoclonal antibody directed against an antigen chosen from the group consisting essentially of: vaccinia H3L, D8L, B5R, A27L and A17L.

6. The composition of claim 4 wherein said composition inhibits vaccinia virus infection in a subject in vivo.

7. The composition of claim 6 wherein said subject is avian or mammalian.

8. The composition of claim 4 wherein said composition ameliorates symptoms of vaccinia virus infection when said composition is administered to a subject after infection with vaccinia virus.

9. The composition of claim 8 wherein said subject is avian or mammalian.

10. The composition of claim 2 wherein said monoclonal antibody immunoprecipitates L1R in vitro.

11. The composition of claim 3 wherein said monoclonal antibody immunoprecipitates A33R in vitro.

12. A therapeutic composition for ameliorating symptoms of vaccinia virus infection comprising the composition of claim 4, and a pharmaceutically acceptable excipient.

13. A passive vaccine against vaccinia virus infection comprising the composition of claim 4.

14. An anti-vaccinia composition, comprising one or more monoclonal antibodies, wherein at least two of said monoclonal antibodies are directed against L1R and A33R, in an amount effective for inhibiting vaccinia virus infection, and a pharmaceutically acceptable carrier.

15. A method of treating vaccinia virus infection comprising administering to a patient in need of said treatment an effective amount of a composition according to claim 4.

16. The composition according to claim 1 wherein said vaccinia virus antigen is chosen from the vaccinia strain Connaught, IHD-J, Brighton, WR, Lister, Copenhagen, Ankara, Dairen I, L-IPV, LC16M8, LC16MO, LIVP, Tian Tan, WR 65-16, Wyeth.

17. A poxvirus monoclonal antibody composition comprising monoclonal antibodies against a homolog of a vaccinia antigen chosen from the group consisting of L1R and A33R, said poxvirus chosen from the group consisting of: orthopoxvirus, parapoxvirus, avipoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, molluscimpoxvirus, and yatapoxvirus.

L8 ANSWER 7 OF 10 USPATFULL on STN
2002:314399 DNA vaccines against poxviruses.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES

Schmaljohn, Connie S., Frederick, MD, UNITED STATES
US 2002176871 A1 20021128
APPLICATION: US 2001-800632 A1 20010307 (9)
PRIORITY: US 2000-187608P 20000307 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In this application is described a poxvirus naked DNA vaccine which protects animals against poxvirus challenge comprising IMV and EEV nucleic acids from poxvirus. Methods of use of the vaccine and its advantages are described.

CLM What is claimed is:

1. A DNA vaccine against poxviruses comprising at least one nucleic acids encoding an intracellular mature virion antigen and at least one nucleic acid encoding an extracellular enveloped virion antigen of a poxvirus.

2. The DNA vaccine of claim 1 wherein said poxvirus is chosen from the group consisting of: variola virus, monkeypox virus, cowpox virus, orf virus, paravaccinia virus, Tanapoxvirus, Yabapoxvirus and Molluscum contagiosum

3. The vaccine of claim 1 wherein said poxvirus is vaccinia.

4. The vaccine of claim 3 wherein said intracellular mature virion antigen is chosen from the group consisting of: L1R and A27L or a homolog thereof.

5. The vaccine of claim 3 wherein said extracellular mature virion antigen is chosen from the group consisting of: A33R and B5R or a homolog thereof.

6. A method for inducing in a subject an immune response against poxvirus infection comprising administering to said subject an immunologically effective amount of at least one nucleic acid encoding an intracellular mature virion antigen and at least one nucleic acid encoding an extracellular mature virion antigen of said poxvirus in an acceptable diluent.

7. A composition of matter comprising a carrier particle; and a DNA sequence coated onto the carrier particle, the DNA sequence comprising a promoter operative in the cells of a mammal and a protein coding region coding for a poxvirus antigen chosen from the group consisting of: IMV or EEV antigen.

8. The composition of claim 7 wherein said IMV poxvirus antigen is selected from the group consisting of L1R and A27L.

9. The composition of claim 7 wherein said EEV posvirus antigen is selected from the group consisting of A33R and B5R.

10. A vaccine comprising a composition of matter according to claim 8 and a composition of matter according to claim 9.

11. A method for inducing a protective immune response to a poxvirus in a mammal, comprising (i) preparing a nucleic acid encoding an antigen of poxvirus operatively linked to a promoter operative in cells of a mammal; (ii) coating the nucleic acid in (i) onto carrier particles; (iii) accelerating the coated carrier particles into epidermal cells of the mammal in vivo; and (iv) detecting a protective immune response in said mammal upon exposure to a poxvirus.

12. The method according to claim 11 wherein the carrier particles are gold.

13. The method according to claim 11 wherein the antigen is chosen from the group consisting of IMV antigen and EEV antigen

14. The method according to claim 11 wherein said poxvirus is VACV.

15. A multivalent vaccine for protection against infection with more than one poxvirus comprising a composition of matter comprising a carrier particle having one or more DNA sequence coated onto the carrier particle, the DNA sequence comprising a promoter operative in the cells of a mammal and a nucleic acid coding for an antigen, said antigen selected from the group consisting of an IMV antigen and an EEV antigen, of a first poxvirus said poxvirus selected from the group consisting of Orthopoxvirus, Parapoxvirus, Caripoxvirus, Suipoxvirus, Leporipoxvirus, Avipoxvirus, Yatapoxvirus, Molluscipoxvirus, macropod poxvirus, and crocodilian poxvirus.

16. The multivalent vaccine of claim 15, further comprising a composition comprising a carrier particle having one or more DNA sequence coated onto the carrier particle, the DNA sequence comprising a promoter operative in the cells of a mammal a nucleic acid coding for an antigen, said antigen selected from the group consisting of IMV antigen and EEV antigen, of a second poxvirus different from said first poxvirus, said second poxvirus selected from the group consisting of Orthopoxvirus, Parapoxvirus, Caripoxvirus, Suipoxvirus, Leporipoxvirus, Avipoxvirus, Yatapoxvirus, Molluscipoxvirus, macropod poxvirus, and crocodilian poxvirus, wherein the nucleic acid coding for an IMV antigen is not on the same carrier particle as the nucleic acid coding for an EEV antigen.

L8 ANSWER 8 OF 10 USPATFULL on STN
2002:294539 EBOLA VIRION PROTEINS EXPRESSED FROM VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS REPLICONS.
HART, MARY K., FREDERICK, MD, UNITED STATES
WILSON, JULIE A., FREDERICK, MD, UNITED STATES
PUSHKO, PETER, FREDERICK, MD, UNITED STATES
SMITH, JONATHAN F., SABILLASVILLE, MD, UNITED STATES
SCHMALJOHN, ALAN L., FREDERICK, MD, UNITED STATES
US 2002164582 A1 20021107
APPLICATION: US 1999-337946 A1 19990622 (9)
PRIORITY: US 1998-91403P 19980629 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Using the Ebola GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is protective against infection with Ebola virus is described.

CLM What is claimed is:

1. A DNA fragment which encodes a GP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:1, or a polynucleotide fragment comprising at least 15 nucleotides.

2. A DNA fragment which encodes a NP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:2, or a polynucleotide fragment comprising at least 15 nucleotides.

3. A DNA fragment which encodes a VP24 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:3, or a polynucleotide fragment comprising at least 15 nucleotides.

4. A DNA fragment which encodes a VP30 Ebola protein, said DNA fragment comprising the sequence specified in any of SEQ ID NO:4 and SEQ ID NO:7, or a polynucleotide fragment comprising at least 15 nucleotides.

5. A DNA fragment which encodes a VP35 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:5, or a polynucleotide fragment comprising at least 15 nucleotides.

6. A DNA fragment which encodes a VP40 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:6, or a polynucleotide fragment comprising at least 15 nucleotides.

7. A DNA fragment which encodes a GP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:17 or a conservative substitution thereof.

8. A DNA fragment which encodes a NP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:18 or a conservative substitution thereof.

9. A DNA fragment which encodes a VP24 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:19 or a conservative substitution thereof.

10. A DNA fragment which encodes a VP30 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in any of SEQ ID NO:20 and SEQ ID NO:23 or a conservative substitution thereof.

11. A DNA fragment which encodes a VP35 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:21 or a conservative substitution thereof.

12. A DNA fragment which encodes a VP40 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in

SEQ ID NO:22 or a conservative substitution thereof.

13. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6 and 7 or a fragment thereof comprising at least 15 nucleotides.

14. A recombinant DNA construct comprising: (i) a vector, and (ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24 and 25 or a conservative substitution thereof.

15. The recombinant DNA construct of claim 13 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

16. The recombinant DNA construct of claim 14 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

17. A recombinant DNA construct according to claim 13 wherein said vector is an expression vector.

18. A recombinant DNA construct according to claim 13 wherein said vector is a prokaryotic vector.

19. A recombinant DNA construct according to claim 13 wherein said vector is a eukaryotic vector.

20. A recombinant DNA construct according to claim 14 wherein said vector is an expression vector.

21. A recombinant DNA construct according to claim 14 wherein said vector is a prokaryotic vector.

22. A recombinant DNA construct according to claim 14 wherein said vector is a eukaryotic vector.

23. The recombinant DNA construct of claim 17 wherein said vector is a VEE virus replicon vector.

24. The recombinant DNA construct of claim 20 wherein said vector is a VEE virus replicon vector.

25. The recombinant DNA construct according to claim 23 wherein said Ebola virus DNA fragments are from Ebola Zaire 1976.

26. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP24.

27. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP30.

28. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP35.

29. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP40.

30. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboNP.

31. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboGP.

32. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboVP30(#2).

33. Self replicating RNA produced from a construct chosen from the group consisting of EboVP24ReP, EboVP30ReP, EboVP35ReP, EboVP40ReP, EboVPNPReP, EboVPGPReP, and EboVP30ReP(#2).

34. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 33.

35. A pharmaceutical composition comprising infectious alphavirus particles according to claim 34 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

36. A host cell transformed with a recombinant DNA construct according to claim 13.

37. A host cell transformed with a recombinant DNA construct according to claim 14.

38. A host cell according to claim 36 wherein said host cell is prokaryotic.

39. A host cell according to claim 36 wherein said host cell is eukaryotic.

40. A host cell according to claim 37 wherein said host cell is prokaryotic.

41. A host cell according to claim 37 wherein said host cell is eukaryotic.

42. A method for producing Ebola virus proteins comprising culturing the cells according to claim 36 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

43. A method for producing Ebola virus proteins comprising culturing the cells according to claim 37 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

44. A method for producing Ebola virus proteins comprising culturing the cells according to claim 38 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

45. A method for producing Ebola virus proteins comprising culturing the cells according to claim 39 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

46. An isolated and purified Ebola GP protein specified in SEQ ID NO:17 and conservative substitutions thereof, or an immunologically identifiable portion thereof.

47. An isolated and purified Ebola NP protein specified in SEQ ID NO:18 and conservative substitutions thereof or an immunologically identifiable portion thereof.

48. An isolated and purified Ebola VP24 protein specified in SEQ ID NO:19 and conservative substitutions thereof or an immunologically identifiable portion thereof.

49. An isolated and purified Ebola VP30 protein specified in any of SEQ ID NO:20 and SEQ ID NO:23 and conservative substitutions thereof or an immunologically identifiable portion thereof.

50. An isolated and purified Ebola VP35 protein specified in SEQ ID NO:21 and conservative substitutions thereof or an immunologically identifiable portion thereof.

51. An isolated and purified Ebola VP40 protein specified in SEQ ID NO:22 and conservative substitutions thereof or an immunologically identifiable portion thereof.

52. An antibody to a peptide encoded by the sequence specified in SEQ ID NO:17, 18, 19, 20, 21, 22, 23, 24, and 25.

53. A method for detecting Ebola virus infection comprising contacting a sample from a subject suspected of having Ebola virus infection with a antibody according to claim 52 and detecting the presence or absence by detecting the presence or absence of a complex formed between the Ebola protein and antibodies specific therefor.

54. A method for detecting the presence or absence of Ebola virus GP RNA in a sample using the polymerase chain reaction using primers for Ebola GP nucleic acid sequence specified in SEQ ID NO:1 for GP.

55. An Ebola infection diagnostic kit comprising at least 12 consecutive nucleotides of SEQ ID NO:1 specific for the amplification of DNA or RNA of Ebola virus in a sample using the polymerase chain reaction and ancillary reagents suitable for use in such a reaction for detecting the presence or absence of Ebola virus DNA or RNA in a sample.

56. A vaccine for Ebola comprising alphavirus particles of claim 34.

57. A method for the diagnosis of Ebola virus infection comprising the steps of: (i) contacting a sample from an individual suspected of

having Ebola virus infection with an antibody to Ebola proteins according to claim 52; and (ii) detecting the presence or absence of Ebola virus infection by detecting the presence or absence of a complex formed between Ebola proteins and antibodies specific therefor.

58. A pharmaceutical composition comprising the self replicating RNA of claim 33 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

59. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of VRepEboVP24, VRepEboVP30, VRepEboVP35, VRepEboVP40, VRepEboNP, VRepEboGP, and VRepEboVP30(#2), in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier and/or adjuvant.

60. A pharmaceutical composition comprising comprising a peptide encoded by any of SEQ ID NO:24 and SEQ ID NO:25, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

L8 ANSWER 9 OF 10 USPATFULL on STN
2002:16571 Prophylactic and therapeutic monoclonal antibodies.
Hooper, Jay W., New Market, MD, UNITED STATES
Schmaljohn, Alan L., Frederick, MD, UNITED STATES
Schmaljohn, Connie S., Frederick, MD, UNITED STATES
US 2002009447 A1 20020124
APPLICATION: US 2001-781124 A1 20010209 (9)
PRIORITY: US 2000-182066P 20000211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB In this application are described vaccinia monoclonal antibodies. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention, and/or therapeutical treatment of vaccinia virus infections in vitro and in vivo.
CLM What is claimed is:
1. A composition comprising one or more monoclonal antibody directed against a vaccinia virus antigen.

2. The composition of claim 1 wherein said vaccinia virus antigen is L1R.

3. The composition of claim 1 wherein said vaccinia virus antigen is A33R.

4. The composition of claim 2 wherein said composition further comprises one or more monoclonal antibody directed against vaccinia A33R.

5. The composition of claim 4 wherein said composition further comprises one or more monoclonal antibody directed against an antigen chosen from the group consisting essentially of: vaccinia H3L, D8L, B5R, A27L and A17L.

6. The composition of claim 4 wherein said composition inhibits vaccinia virus infection in a subject in vivo.

7. The composition of claim 6 wherein said subject is avian or mammalian.

8. The composition of claim 4 wherein said composition ameliorates symptoms of vaccinia virus infection when said composition is administered to a subject after infection with vaccinia virus.

9. The composition of claim 8 wherein said subject is avian or mammalian.

10. The composition of claim 2 wherein said monoclonal antibody immunoprecipitates L1R in vitro.

11. The composition of claim 3 wherein said monoclonal antibody immunoprecipitates A33R in vitro.

12. A therapeutic composition for ameliorating symptoms of vaccinia virus infection comprising the composition of claim 4, and a pharmaceutically acceptable excipient.

13. A passive vaccine against vaccinia virus infection comprising the composition of claim 4.

14. An anti-vaccinia composition, comprising one or more monoclonal antibodies, wherein at least two of said monoclonal antibodies are directed against L1R and A33R, in an amount effective for inhibiting vaccinia virus infection, and a pharmaceutically acceptable carrier.

15. A method of treating vaccinia virus infection comprising administering to a patient in need of said treatment an effective amount of a composition according to claim 4.

16. The composition according to claim 1 wherein said vaccinia virus antigen is chosen from the vaccinia strain Connaught, IHD-J, Brighton, WR, Lister, Copenhagen, Ankara, Dairen I, L-IPV, LC16M8, LC16MO, LIVP, Tian Tan, WR 65-16, Wyeth.

17. A poxvirus monoclonal antibody composition comprising monoclonal antibodies against a homolog of a vaccinia antigen chosen from the group consisting of L1R and A33R, said poxvirus chosen from the group consisting of: orthopoxvirus, parapoxvirus, avipoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, molluscimpoxvirus, and yatapoxvirus.

L8 ANSWER 10 OF 10 USPATFULL on STN
2001:36806 Genetic induction of anti-viral immune response and genetic vaccine for filovirus.
Haynes, Joel R., Fort Collins, CO, United States
Schmaljohn, Connie S., Frederick, MD, United States
Fuller, Deborah L., Oregon, WI, United States
Schmaljohn, **Alan**, Frederick, MD, United States
Jahrling, Peter B., Middletown, MD, United States
PowerJect Vaccines Inc., Madison, WI, United States (U.S. corporation)
US 6200959 B1 20010313
APPLICATION: US 1996-760615 19961204 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An approach to genetic vaccine methodology is described. A genetic construction encoding antigenic determinants of a filovirus is transfected into cells of the vaccinated individuals using a particle acceleration protocol so as to express the viral antigens in healthy cells to produce an immune response to those antigens.
CLM What is claimed is:
1. A method of inducing an immune response to a Marburg or Ebola virus glycoprotein in a mammal, said method comprising: (a) providing a genetic construction comprising a promoter operative in cells of the mammal and a coding region for a determinant of the glycoprotein, the genetic construction not comprising sequences necessary for replication of the virus; (b) coating copies of the genetic construction onto carrier particles small in size in relation to the size of the cells of the mammal; and (c) accelerating the coated carrier particles into epidermal cells of the mammal in vivo, thereby inducing an immune response against the glycoprotein.

2. A method as claimed in claim 1 wherein the carrier particles are accelerated by a gaseous pulse in order to accelerate the carrier particles toward the mammal.

3. A method as claimed in claim 1 wherein the protein coding region encodes a glycoprotein selected from the group consisting of Ebola Zaire virus gp125, Marburg Musoke virus gp170,and Marburg Ravn virus glycoprotein.

4. A method as claimed in claim 1 wherein the protein coding region comprises SEQ ID NO: 1.

5. A method as claimed in claim 1 wherein the protein coding region comprises SEQ ID NO: 3.

6. A method as claimed in claim 1 wherein the protein coding region comprises SEQ ID NO: 5.

7. A composition of matter comprising a carrier particle and a genetic construction coated onto the carrier particle, wherein the genetic construction comprises a promoter operative in the cells of a mammal and a coding region for a determinant of a Marburg or Ebola virus glycoprotein.

8. A composition as claimed in claim 7 wherein the protein coding region encodes a glycoprotein selected from the group consisting of Ebola Zaire virus gp125, Marburg Musoke virus gp170, and Marburg Ravn virus

glycoprotein.

9. A composition as claimed in claim 7 wherein the protein coding region comprises SEQ ID NO: 1.

10. A composition as claimed in claim 7 wherein the protein coding region comprises SEQ ID NO: 3.

11. A composition as claimed in claim 7 wherein the protein coding region comprises SEQ ID No. 5.

```
=> d his

     (FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005)

     FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
L1          2139 S (FILOVIR? OR MARBURG OR EBOLA)
L2            35 S L1 AND (GP1 OR GP2)
L3           255 S L1 AND (BIVALENT OR MULTIVALENT)
L4             8 S L3 AND (MARBURG/TI OR EBOLA/TI OR FILOVIR?/TI)
L5             6 S L4 NOT L2
                 E GROGAN CASE C/IN
L6             1 S E3
                 E SCHMALJOHN ALAN L/IN
L7            11 S E2 OR E3
L8            10 S L7 NOT L6

=> file wpids
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               131.96     132.38

FILE 'WPIDS' ENTERED AT 18:38:30 ON 24 JUL 2005
COPYRIGHT (C) 2005 THE THOMSON CORPORATION

FILE LAST UPDATED:          20 JUL 2005      <20050720/UP>
MOST RECENT DERWENT UPDATE:     200546        <200546/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE
    http://thomsonderwent.com/coverage/latestupdates/           <<<

>>> FOR INFORMATION ON ALL DERWENT WORLD PATENTS INDEX USER
    GUIDES, PLEASE VISIT:
    http://thomsonderwent.com/support/userguides/               <<<

>>> NEW! FAST-ALERTING ACCESS TO NEWLY-PUBLISHED PATENT
    DOCUMENTATION NOW AVAILABLE IN DERWENT WORLD PATENTS INDEX
    FIRST VIEW - FILE WPIFV.
    FOR FURTHER DETAILS: http://www.thomsonderwent.com/dwpifv  <<<

>>> THE CPI AND EPI MANUAL CODES HAVE BEEN REVISED FROM UPDATE 200501.
    PLEASE CHECK:
http://thomsonderwent.com/support/dwpiref/reftools/classification/code-revision/
    FOR DETAILS. <<<

=> s (filovir? or marburg or ebola)
           118 FILOVIR?
            95 MARBURG
           232 EBOLA
L9         329 (FILOVIR? OR MARBURG OR EBOLA)

=> s l9 and (multivalent or bivalent)
          3912 MULTIVALENT
         10065 BIVALENT
L10          5 L9 AND (MULTIVALENT OR BIVALENT)

=> d l10,ti,1-5

L10  ANSWER 1 OF 5  WPIDS  COPYRIGHT 2005 THE THOMSON CORP on STN
TI   New peptides that specifically target and bind to dendritic cells (e.g.
     myeloid, Langerhans or plasmacytoid dendritic cells), useful for promoting
     an immune response against pathogenic viruses (e.g. HIV) or tumor cells.
```

L10 ANSWER 2 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Compound useful for treating e.g. cancer comprises optionally stabilized nucleic acid, aptamer, antisense sequence, or antisense mimic conjugated to a ligand for the transcobalamin receptor or intrinsic factor receptor.

L10 ANSWER 3 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI New chimeric **filovirus** glycoprotein (GP) protein comprising GP1 and GP2, useful for inducing an immune response against infection of different **filoviruses**, specifically against both **Ebola** and **Marburg** viruses.

L10 ANSWER 4 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Preparing DNA for preparing viral RNA or virions, involves cloning DNA having full length copy of genomic RNA of RNA virus, or its fragment encoding RNA dependent RNA polymerase into bacterial artificial chromosome.

L10 ANSWER 5 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Vaccine comprising immunogenic peptide - for prevention and treatment of simian herpes virus B infection.

=> d l10,bib,ab,3

L10 ANSWER 3 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2003-040651 [03] WPIDS
DNC C2003-009637
TI New chimeric **filovirus** glycoprotein (GP) protein comprising GP1 and GP2, useful for inducing an immune response against infection of different **filoviruses**, specifically against both **Ebola** and **Marburg** viruses.
DC B04 D16
IN GROGAN, C C; HEVEY, M C; SCHMALJOHN, A L
PA (GROG-I) GROGAN C C; (HEVE-I) HEVEY M C; (SCHM-I) SCHMALJOHN A L; (USSA) US ARMY MEDICAL RES INST INFECTIOUS DISE
CYC 84
PI WO 2002079239 A2 20021010 (200303)* EN 94
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
US 2003108560 A1 20030612 (200340)
AU 2002303086 A1 20021015 (200432)
ADT WO 2002079239 A2 WO 2002-US3339 20020131; US 2003108560 A1 Provisional US 2001-267522P 20010131, US 2002-66506 20020131; AU 2002303086 A1 AU 2002-303086 20020131
FDT AU 2002303086 A1 Based on WO 2002079239
PRAI US 2001-267522P 20010131; US 2002-66506 20020131
AB WO 200279239 A UPAB: 20030113
NOVELTY - A chimeric **filovirus** glycoprotein (GP) protein comprising GP1 and GP2, where GP1 is from a **filovirus** different than that of GP2, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a DNA fragment encoding a chimeric protein above and comprising a sequence of 2252, 1841, 2046 or 2246 bp given in the specification, or their conservative substitutions;

(2) a recombinant DNA construct comprising a vector, and a DNA fragment encoding a chimeric **filovirus** GP protein defined above;

(3) self replicating RNA produced from the construct of (2);

(4) infectious alphavirus particles produced from packaging the self replicating RNA of (3);

(5) a pharmaceutical composition comprising infectious alphavirus particles in a pharmaceutical carrier and/or adjuvant;

(6) a host cell transformed with a recombinant DNA construct;

(7) producing chimeric **filovirus** GP proteins by culturing the cells under conditions such that the DNA fragment is expressed and the chimeric protein is produced;

(8) a vaccine for more than one **filovirus** comprising viral particles containing one or more replicon RNA encoding chimeric GP from one or more **filovirus**;

(9) vaccines against **Ebola** and **Marburg** virus infections comprising a chimeric GP protein above, of infectious alphavirus particles produced from replicating RNA produced from the construct above;

(10) a pharmaceutical composition comprising a chimeric peptide encoded by any of the DNA sequences above, in a pharmaceutical carrier and/or adjuvant;

(11) a **bivalent filovirus** vaccine antigen comprising a chimeric GP protein comprising GP1 or its portion, from a first **filovirus** and GP2 or its portion from a second **filovirus**, where the antigen is able to elicit an immune response to 2 **filoviruses** in a subject; and

(12) a **multivalent filovirus** vaccine antigen comprising a chimeric GP protein where GP1 and GP2 are comprised of portions of GP1 and GP2 from different **filoviruses**, where the antigen able to elicit an immune response to more than 2 **filoviruses** in a subject.

ACTIVITY - Virucide. No biological data is given.

MECHANISM OF ACTION - Vaccine.

USE - The chimeric **filovirus** GP protein is useful for inducing an immune response against infection of different **filoviruses**, specifically against both **Ebola** and **Marburg** viruses.

ADVANTAGE - The single-component **bivalent** vaccine comprising the chimeric **filovirus** GP protein is cost-effective, easy to produce, develop and test, and provides a protective immune response to multiple **filovirus** agents in a single component.

Dwg.0/10

=> file medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	11.70	144.08

FILE 'MEDLINE' ENTERED AT 18:39:46 ON 24 JUL 2005

FILE LAST UPDATED: 23 JUL 2005 (20050723/UP). FILE COVERS 1950 TO DATE.

On December 19, 2004, the 2005 MeSH terms were loaded.

The MEDLINE reload for 2005 is now available. For details enter HELP RLOAD at an arrow promt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

OLDMEDLINE now back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2005 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> file uspatful

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.38	144.46

FILE 'USPATFULL' ENTERED AT 18:39:53 ON 24 JUL 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Jul 2005 (20050721/PD)
FILE LAST UPDATED: 21 Jul 2005 (20050721/ED)
HIGHEST GRANTED PATENT NUMBER: US6920641
HIGHEST APPLICATION PUBLICATION NUMBER: US2005160510
CA INDEXING IS CURRENT THROUGH 21 Jul 2005 (20050721/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 21 Jul 2005 (20050721/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2005

>>> USPAT2 is now available. USPATFULL contains full text of the original, i.e., the earliest published granted patents or applications. USPAT2 contains full text of the latest US publications, starting in 2001, for the inventions covered in USPATFULL. A USPATFULL record contains not only the original published document but also a list of any subsequent publications. The publication number, patent kind code, and publication date for all the US publications for an invention are displayed in the PI (Patent Information) field of USPATFULL records and may be searched in standard search fields, e.g., /PN, /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together through the new cluster USPATALL. Type FILE USPATALL to enter this cluster. <<<

>>> <<<

>>> Use USPATALL when searching terms such as patent assignees, <<<

>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> s dengue virus?
          2292 DENGUE
        106600 VIRUS?
L11       1167 DENGUE VIRUS?
                 (DENGUE(W)VIRUS?)

=> s l11 and (gp or glycoprotein?)
         15528 GP
         38708 GLYCOPROTEIN?
L12        696 L11 AND (GP OR GLYCOPROTEIN?)

=> s l12 and (bivalent or multivalent)
         18249 BIVALENT
         13053 MULTIVALENT
L13        149 L12 AND (BIVALENT OR MULTIVALENT)

=> s l13 and chimeric
         40052 CHIMERIC
L14        106 L13 AND CHIMERIC

=> s l14 and chimeric/clm
          7101 CHIMERIC/CLM
L15         22 L14 AND CHIMERIC/CLM

=> d l15,cbib,ab,clm,1-22

L15  ANSWER 1 OF 22  USPATFULL on STN
2005:144275 Whole cell engineering by mutagenizing a substantial portion of a
    starting genome combining mutations and optionally repeating.
    Short, Jay M, Rancho Santa Fe, CA, UNITED STATES
    Fu, Pengcheng, Lowrey Avenue, HI, UNITED STATES
    Wei, Jing, San Diego, CA, UNITED STATES
    Levin, Michael, San Diego, CA, UNITED STATES
    Latterich, Martin, Montellano Terrace, San Diego, CA, UNITED STATES
    US 2005124010 A1 20050609
    APPLICATION: US 2003-398271  A1  20011001 (10)
    WO 2001-US31004 20011001
    PRIORITY: US 2003-9677584  20000930
    US 2003-279702P  20010328 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     This invention relates to the field of cellular and whole organism
       engineering. Specifically, this invention relates to a cellular
       transformation, directed evolution, and screening method for creating
       novel transgenic organisms having desirable properties. Thus in one
       aspect, this invention relates to a method of generating a transgenic
       organism, such as a microbe or a plant, having a plurality of traits
       that are diffenentially activatable.
CLM    What is claimed is:
       1. A method for identifying proteins by differential labeling of
       peptides, the method comprising the following steps: (a) providing a
       sample comprising a polypeptide; (b) providing a plurality of labeling
       reagents which differ in molecular mass that can generate differential
       labeled peptides that do not differ in chromatographic retention
       properties and do not differ in ionization and detection properties in
       mass spectrographic analysis, wherein the differences in molecular mass
       are distinguishable by mass spectrographic analysis; (c) fragmenting the
       polypeptide into peptide fragments by enzymatic digestion or by
       non-enzymatic fragmentation; (d) contacting the labeling reagents of
       step (b) with the peptide fragments of step (c), thereby labeling the
       peptides with the differential labeling reagents; (e) separating the
       peptides by chromatography to generate an eluate; (f) feeding the
       eluate of step (e) into a mass spectrometer and quantifying the amount
       of each peptide and generating the sequence of each peptide by use of
       the mass spectrometer; (g) inputting the sequence to a computer program
       product which compares the inputted sequence to a database of
       polypeptide sequences to identify the polypeptide from which the
       sequenced peptide originated.

       2. The method of claim 1, wherein the sample of step (a) comprises a
       cell or a cell extract.

       3. The method of claim 1, further comprising providing two or more
```

samples comprising a polypeptide.

4. The method of claim 3, wherein one sample is derived from a wild type cell and one sample is derived from an abnormal or a modified cell.

5. The method of claim 4, wherein the abnormal cell is a cancer cell.

6. The method of claim 1, further comprising purifying or fractionating the polypeptide before the fragmenting of step (c).

7. The method of claim 1, further comprising purifying or fractionating the polypeptide before the labeling of step (d).

8. The method of claim 1, further comprising purifying or fractionating the labeled peptide before the chromatography of step (e).

9. The method of claim 6, claim 8 or claim 8, wherein the purifying or fractionating comprises a method selected from the group consisting of size exclusion chromatography, size exclusion chromatography, HPLC, reverse phase HPLC and affinity purification.

10. The method of claim 1, further comprising contacting the polypeptide with a labeling reagent of step (b) before the fragmenting of step (c).

11. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of: i. Z^{AOH} and Z^{BOH}, to esterify peptide C-terminals and/or Glu and Asp side chains; ii. Z^{ANH_2} and Z^{BNH_2}, to form amide bond with peptide C-terminals and/or Glu and Asp side chains; and iii. $Z^{ACO_{2H}}$ and $Z^{BCO_{2H}}$. to form amide bond with peptide N-terminals and/or Lys and Arg side chains; wherein Z^A and Z^B independently of one another comprise the general formula $R\text{-}Z^1\text{-}A1\text{-}Z2\text{-}A2\text{-}Z3\text{-}A3\text{-}Z4\text{-}A4\text{-}$, Z^1, Z^2, Z^3, and Z^4 independently of one another, are selected from the group consisting of nothing, O, OC(O), OC(S), OC(O)O, OC(O)NR, OC(S)NR, OSiRR^1, S, SC(O), SC(S), SS, S(O), S(O_2), NR, NRR^1+, C(O), C(O)O, C(S), C(S)O, C(O)S, C(O)NR, C(S)NR, SiRR^1, $(Si(RR^1)O)_n$, SnRR^1, Sn(RR^1)O, BR(OR^1), BRR^1, B(OR)(OR^1), OBR(OR^1, OBRR^1, and OB(OR)(OR^1), and R and R^1 is an alkyl group, A^1, A^2, A^3, and A^4 independently of one another, are selected from the group consisting of nothing or $(CRR^1)_n$, wherein R, R^1, independently from other R and R^1 in Z^1 to Z^4 and independently from other R and R^1 in A^1 to A^4, are selected from the group consisting of a hydrogen atom, a halogen atom and an alkyl group; n in $Z^1$to Z^4, independent of n in A^1 to A^4, is an integer having a value selected from the group consisting of 0 to about 51; 0 to about 41; 0 to about 31; 0 to about 21, 0 to about 11 and 0 to about 6.

12. The method of claim 11, wherein the alkyl group is selected from the group consisting of an alkenyl, an alkynyl and an aryl group.

13. The method of claim 11, wherein one or more C--C bonds from (CRR^1)n are replaced with a double or a triple bond,

14. The method of claim 13, wherein an R or an R^1 group is deleted.

15. The method of claim 13, wherein (CRR^1)n is selected from the group consisting of an o-arylene, an m-arylene and a p-arylene, wherein each group has none or up to 6 substituents.

16. The method of claim 13, wherein (CRR^1)n is selected from the group consisting of a carbocyclic, a bicyclic and a tricyclic fragment, wherein the fragment has up to 8 atoms in the cycle with or without a heteroatom selected from the group consisting of an O atom, a N atom and an S atom.

17. The method of claim 1, wherein two or more labeling reagents have the same structure but a different isotope composition.

18. The method of claim 11, wherein Z^A has the same structure as Z^B, but Z^A has a different isotope composition than Z^B.

19. The method of claim 17, wherein the isotope is boron-10 and boron-11.

20. The method of claim 17, wherein the isotope is carbon-12 and carbon-13.

21. The method of claim 17, wherein the isotope is nitrogen-14 and nitrogen-15.

22. The method of claim 17, wherein the isotope is sulfur-32 and sulfur-34.

23. The method of claim 17, wherein, where the isotope with the lower mass is x and the isotope with the higher mass is y, and x and y are integers, x is greater than y.

24. The method of claim 17, wherein x and y are between 1 and about 11, between 1 and about 21, between 1 and about 31, between 1 and about 41, or between 1 and about 51.

25. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of: i. $CD_3(CD_2)_{nOH/CH3}(CH_2)_{nOH}$, to esterify peptide C-terminals, where n=0, 1, 2 or y; ii. $CD_3(CD_2)_{nNH2}$ $CH_3(CH_2)_{nNH2}$, to form amide bond with peptide C-terminals, where n=0, 1, 2 or y; and iii. $D(CD_2)_{nCO2H/H}(CH_2)_{nCO2H}$, to form amide bond with peptide N-terminals, where n=0, 1, 2 or y; wherein D is a deuteron atom, and y is an integer selected from the group consisting of about 51; about 41; about 31; about 21, about 11; about 6 and between about 5 and 51.

26. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of: i. Z^{AOH} and Z^{BOH} to esterify peptide C-terminals; ii. $Z^{ANH_2/}Z^{BNH_2}$ to form an amide bond with peptide C-terminals; and iii. $Z^{ACO_{2H}/}Z^{BCO_{2H}}$ to form an amide bond with peptide N-terminals; wherein Z^A and Z^B have the general formula $R\text{-}Z^{1\text{-}A1\text{-}Z2\text{-}A2\text{-}Z3\text{-}A3\text{-}Z4\text{-}A4\text{-}Z1}$, Z^2, Z^3, and Z^4, independently of one another, are selected from the group consisting of nothing, O, OC(O), OC(S), OC(O)O, OC(O)NR, OC(S)NR, $OSiRR^1$, S, SC(O), SC(S), SS, S(O), $S(O_2)$, NR, NRR^1+, C(O), C(O)O, C(S), C(S)O, C(O)S, C(O)NR, C(S)NR, $SiRR^1$, $(Si(RR^1)O)_n$, $SnRR^1$, $Sn(RR^1)O$, $BR(OR^1)$, BRR^1, $B(OR)(OR^1)$, $OBR(OR^1)$, $OBRR^1$, and $OB(OR)(OR^1)$; A^1, A^2, A^3, and A^4, independently of one another, are selected from the group consisting of nothing and the general formulae $(CRR^1)_n$, and, R and R^1 is an alkyl group.

27. The method of claim 26, wherein a single C--C bond in a $(CRR^1)_n$ group is replaced with a double or a triple bond.

28. The method of claim 27, wherein R and R^1 are absent.

29. The method of claim 27, wherein $(CRR^1.)_n$ comprises a moiety selected from the group consisting of an o-arylene, an m-arylene and ap-arylene, wherein the group has none or up to 6 substituents.

30. The method of claim 27, wherein the group comprises a carbocyclic, a bicyclic, or a tricyclic fragments with up to 8 atoms in the cycle, with or without a heteroatom selected from the group consisting of an O atom, an N atom and an S atom.

31. The method of claim 26, wherein R, R^1, independently from other R and R^1 in $Z^{1\text{-}Z4}$ and independently from other R and R^1 in $A^{1\text{-}A4}$, are selected from the group consisting of a hydrogen atom, a halogen and an alkyl group.

32. The method of claim 31, wherein the alkyl group is selected from the group consisting of an alkenyl, an alkynyl and an aryl group.

33. The method of claim 26, wherein n in $Z^{1\text{-}Z4}$ is independent of n in $A^{1\text{-}A4}$ and is an integer selected from the group consisting of about 51; about 41; about 31; about 21, about 11 and about 6.

34. The method of claim 26, wherein Z^A has the same structure a Z^B but Z^A further comprises x number of $--CH_2--$ fragment(s) in one or more $A^{1\text{-}A4}$ fragments, wherein x is an integer.

35. The method of claim 26, wherein Z^A has the same structure a

Z^B but Z^A further comprises x number of --CF_2-- fragment(s) in one or more A^{1-A4} fragments, wherein x is an integer.

36. The method of claim 26, wherein Z^A comprises x number of protons and Z^B comprises y number of halogens in the place of protons, wherein x and y are integers.

37. The method of claim 26, wherein Z^A contains x number of protons and Z^B contains y number of halogens, and there are x-y number of protons remaining in one or more A^{1-A4} fragments, wherein x and y are integers

38. The method of claim 26, wherein Z^A further comprises x number of-O-- fragment(s) in one or more A^{1-A4} fragments, wherein x is an integer.

39. The method of claim 26, wherein Z^A further comprises x number of --S-- fragment(s) in one or more A^{1-A4} fragments, wherein x is an integer.

40. The method of claim 26, wherein Z^A further comprises x number of --O-- fragment(s) and Z^B further comprises y number of --S-- fragment(s) in the place of --O-- fragment(s), wherein x and y are integers.

41. The method of claim 26, wherein Z^A further comprises x-y number of --O-- fragment(s) in one or more A^{1-A4} fragments, wherein x and y are integers.

42. The method of claim 37, claim 40 or claim 41, wherein x and y are integers selected from the group consisting of between 1 about 51; between 1 about 41; between 1 about 31; between 1 about 21, between 1 about 11 and between 1 about 6, wherein x is greater than y.

43. The method of claim 1, wherein the labeling reagent of step (b) comprises the general formulae selected from the group consisting of: i. $CH_3(CH_2)_{nOH/CH3}(CH_2)_n+mOH$, to esterify peptide C-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . y; ii. $CH_3(CH_2)_n\ NH_{2/CH3}(CH_2)_n+mNH_2$, to form amide bond with peptide C-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . , y; and, iii. $H(CH_2)_{nCO2H/H}(CH_2)$.sub .n+mCO_{2H}, to form amide bond with peptide N-terminals, where n=0, 1, 2, . . . , y; m=1, 2, . . . , y; wherein n, m and y are integers.

44. The method of claim 43, wherein n, m and y are integers selected from the group consisting of about 51; about 41; about 31; about 21, about 11; about 6 and between about 5 and 51.

45. The method of claim 1, wherein the separating of step (e) comprises a liquid chromatography system.

46. The method of claim 1, wherein the liquid chromatography system comprises a multidimensional liquid chromatography.

47. The method of claim 1, wherein the mass spectrometer comprises a tandem mass spectrometry device.

48. The method of claim 1, further comprising quantifying the amount of each polypeptide.

49. The method of claim 1, further comprising quantifying the amount of each peptide.

50. A method for defining the expressed proteins associated with a given cellular state, the method comprising the following steps: (a) providing a sample comprising a cell in the desired cellular state; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting polypeptides derived from the cell into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents; (e) separating the peptides by chromatography to generate an eluate; (f) feeding the eluate of step (e) into a mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of

the mass spectrometer; (g) inputting the sequence to a computer program product which compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated, thereby defining the expressed proteins associated with the cellular state.

51. A method for quantifying changes in protein expression between at least two cellular states, the method comprising the following steps: state; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting polypeptides derived from the cells into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents, wherein the labels used in one same are different from the labels used in other samples; (e) separating the peptides by chromatography to generate an eluate; (f) feeding the eluate of step (e) into a mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program product which identifies from which sample each peptide was derived, compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated, and compares the amount of each polypeptide in each sample, thereby quantifying changes in protein expression between at least two cellular states.

52. A method for identifying proteins by differential labeling of peptides, the method comprising the following steps: (a) providing a sample comprising a polypeptide; (b) providing a plurality of labeling reagents which differ in molecular mass but do not differ in chromatographic retention properties and do not differ in ionization and detection properties in mass spectrographic analysis, wherein the differences in molecular mass are distinguishable by mass spectrographic analysis; (c) fragmenting the polypeptide into peptide fragments by enzymatic digestion or by non-enzymatic fragmentation; (d) contacting the labeling reagents of step (b) with the peptide fragments of step (c), thereby labeling the peptides with the differential labeling reagents; (e) separating the peptides by multidimensional liquid chromatography to generate an eluate; (f) feeding the eluate of step (e) into a tandem mass spectrometer and quantifying the amount of each peptide and generating the sequence of each peptide by use of the mass spectrometer; (g) inputting the sequence to a computer program product which compares the inputted sequence to a database of polypeptide sequences to identify the polypeptide from which the sequenced peptide originated.

53. A **chimeric** labeling reagent comprising (a) a first domain comprising a biotin; and (b) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the **chimeric** labeling reagent comprises at least one isotope.

54. The **chimeric** labeling reagent of claim 53, wherein the isotope is in the first domain.

55. The **chimeric** labeling reagent of claim 54, wherein the isotope is in the biotin.

56. The **chimeric** labeling reagent of claim 53, wherein the isotope is in the second domain.

57. The **chimeric** labeling reagent of claim 53, wherein the isotope is selected from the group consisting of a deuterium isotope, a boron-10 or boron-11 isotope, a carbon-12 or a carbon-13 isotope, a nitrogen-14 or a nitrogen-15 isotope and a sulfur-32 or a sulfur-34 isotope.

58. The **chimeric** labeling reagent of claim 53 comprising two or more isotopes.

59. The **chimeric** labeling reagent of claim 53, wherein the reactive group capable of covalently binding to an amino acid is selected from the group consisting of a succimide group, an isothiocyanate group and an isocyanate group.

60. The **chimeric** labeling reagent of claim 53, wherein the reactive group capable of covalently binding to an amino acid binds to a lysine or a cysteine.

61. The **chimeric** labeling reagent of claim 53, further comprising a linker moiety linking the biotin group and the reactive group.

62. The **chimeric** labeling reagent of claim 53; wherein the linker moiety comprises at least one isotope.

63. The **chimeric** labeling reagent of claim 53, wherein the linker is a cleavable moiety.

64. The **chimeric** labeling reagent of claim 53, wherein the linker can be cleaved by enzymatic digest.

65. The **chimeric** labeling reagent of claim 53, wherein the linker can be cleaved by reduction.

66. A method of comparing relative protein concentrations in a sample comprising (a) providing a plurality of differential small molecule tags, wherein the small molecule tags are structurally identical but differ in their isotope composition, and the small molecules comprise reactive groups that covalently bind to cysteine or lysine residues or both; (b) providing at least two samples comprising polypeptides; (c) attaching covalently the differential small molecule tags to amino acids of the polypeptides; (d) determining the protein concentrations of each sample in a tandem mass spectrometer; and, (d) comparing relative protein concentrations of each sample.

67. The method of claim 66, wherein the sample comprises a complete or a fractionated cellular sample.

68. The method of claim 66, wherein differential small molecule tags comprise a **chimeric** labeling reagent comprising (a) a first domain comprising a biotin; and, (b) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the **chimeric** labeling reagent comprises at least one isotope.

69. The method of claim 68, wherein the isotope is selected from the group consisting of a deuterium isotope, a boron-10 or boron-lI 1 isotope, a carbon-12 or a carbon-13 isotope, a nitrogen-14 or a nitrogen-15 isotope and a sulfur-32 or a sulfur-34 isotope.

70. The method of claim 68, wherein the **chimeric** labeling reagent comprises two or more isotopes.

71. The method of claim 68, wherein the reactive group capable of covalently binding to an amino acid is selected from the group consisting of a succimide group, an isothiocyanate group and an isocyanate group.

72. A method of comparing relative protein concentrations in a sample comprising (a) providing a plurality of differential small molecule tags, wherein the differential small molecule tags comprise a **chimeric** labeling reagent comprising (i) a first domain comprising a biotin; and, (ii) a second domain comprising a reactive group capable of covalently binding to an amino acid, wherein the **chimeric** labeling reagent comprises at least one isotope; (b) providing at least two samples comprising polypeptides; (c) attaching covalently the differential small molecule tags to amino acids of the polypeptides; (d) isolating the tagged polypeptides on a biotin-binding column by binding tagged polypeptides to the column, washing non-bound materials off the column, and eluting tagged polypeptides off the column; (e) determining the protein concentrations of each sample in a tandem mass spectrometer; and, (f) comparing relative protein concentrations of each sample.

73. A method of producing an improved organism having a desirable trait comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations, and c) detecting the presence of said improved organism.

74. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 15 different genes.

75. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 50 different genes.

76. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of a knocking out of at least 100 different genes.

77. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an introduction of at least 15 different genes.

78. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an introduction of at least 50 different genes.

79. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an introduction of at least 100 different genes.

80. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 15 different genes.

81. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 50 different genes.

82. The method of claim 73, wherein the set of substantial genetic mutations in step b) is comprised of an alteration in the expression of at least 100 different genes.

83. A method of producing an improved organism having a desirable trait comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms each having at least one genetic mutation, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations c) detecting the manifestation of at least two genetic mutations, d) introducing at least two detected genetic mutations into one organism, and e) optionally repeating any of steps a), b), c), and d).

84. The method of claim 83, wherein step d) is comprised of a knocking out of at least 15 different genes in one organism.

85. The method of claim 83, wherein step d) is comprised of a knocking out of at least 50 different genes in one organism.

86. The method of claim 83, wherein step d) is comprised of a knocking out of at least 100 different genes in one organism.

87. The method of claim 83, wherein step d) is comprised of an introduction of at least 15 different genes into one organism.

88. The method of claim 83, wherein step d) is comprised of an introduction of at least 50 different genes into one organism.

89. The method of claim 83, wherein step d) is comprised of an introduction of at least 100 different genes into one organism.

90. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 15 different genes in one organism.

91. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 50 different genes in one organism.

92. The method of claim 83, wherein step d) is comprised of an alteration in the expression of at least 100 different genes in one organism.

93. A method for identifying a gene that alters a trait of an organism, comprising: a) obtaining an initial population of organisms, b) generating a set of mutagenized organisms, such that when all the genetic mutations in the set of mutagenized organisms are taken as a whole, there is represented a set of substantial genetic mutations, and c) detecting the presence an organism having said altered trait, and d) determining the nucleotide sequence of a gene that has been mutagenized in the organism having the altered trait.

94. A method for producing an organism with an improved trait, comprising: a) functionally knocking out an enogenous gene in a

substantially clonal population of organisms; b) transferring a library of altered genes into the substantially clonal population of organisms, wherein each altered gene differs from the endogenous gene at only one codon; c) detecting a mutagenized organism having an improved trait; and d) determining the nucleotide sequence of an gene that has been transferred into the detected organism.

95. A method of introducing differentially activatable stacked traits into a transgenic cell or organism, which method is comprised of the following steps: a) obtaining an initial cell or organism; b) introducing into the working cell or organism a plurality of traits (stacked traits), including selectively and differentially activatable traits, whereby serviceable traits for this purpose include traits conferred by genes and traits conferred by gene pathways; c) analyzing the information obtained from steps a) and b), and d) optionally repeating any number or all of the steps of a), b), c), and d);

96. The method of claim 95, wherein step a) also includes holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, **glycoproteins**, or lipids)

97. The method of claim 95, wherein step d) also includes holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, **glycoproteins**, or lipids)

98. The method of claim 95, wherein step a) and d) include holistic monitoring of the strain or organism whereby holistic monitoring can include the detection and/or measurement of all detectable functions and physical parameters (such as but not limited to morphology, behavior, growth, responsiveness to stimuli [e.g., antibiotics, different environment, etc.], and profiles of all detectable molecules, including molecules that are chemically at least in part a nucleic acids, proteins, carbohydrates, proteoglycans, **glycoproteins**, or lipids)

99. The method of claim 95, wherein step b) includes the introduction of at least 15 stacked traits

100. The method of claim 95, wherein step b) includes the introduction of at least 50 stacked traits

101. The method of claim 95, wherein step b) includes the introduction of at least 100 stacked traits

102. The method of claim 96, wherein step a) includes screening cellular characteristics by utilizing one or any combination of the following methods: a) genomics; b) transcriptome characterization or RNA profiling; c) proteomics; d) metabolomics or the analysis of metabolites; e) lipidomics or lipid profiling.

103. A method of claim 102, wherein proteomics specifically includes the use of amino acid reactive tags

104. A method of claim 97, wherein step d) includes screening cellular characteristics by utilizing one or any combination of the following methods: f) genomics; g) transcriptome characterization or RNA profiling; h) proteomics; i) metabolomics or the analysis of metabolites; j) lipidomics or lipid profiling.

105. A method of claim 104, wherein proteomics specifically includes the use of amino acid reactive tags

106. A method of claim 98, wherein steps a) and d) include screening cellular characteristics by utilizing one or any combination of the following methods: k) genomics; l) transcriptome characterization or RNA profiling; m) proteomics; n) metabolomics or the analysis of metabolites; o) lipidomics or lipid profiling. P)

107. A method of claim 106, wherein proteomics specifically includes the use of amino acid reactive tags

108. A method of claim 73, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: q) genomics; r) transcriptome characterization or RNA profiling; s) proteomics; t) metabolomics or the analysis of metabolites; u) lipidomics or lipid profiling.

109. A method of claim 108, wherein proteomics specifically includes the use of amino acid reactive tags

110. A method of claim 93, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: v) genomics; w) transcriptome characterization or RNA profiling; x) proteomics; y) metabolomics or the analysis of metabolites; z) lipidomics or lipid profiling.

111. A method of claim 110, wherein proteomics specifically includes the use of amino acid reactive tags

112. A method of claim 94, wherein step c) includes screening cellular characteristics by utilizing one or any combination of the following methods: aa) genomics; bb) transcriptome characterization or RNA profiling; cc) proteomics; dd) metabolomics or the analysis of metabolites; ee) lipidomics or lipid profiling.

113. A method of claim 112, wherein proteomics specifically includes the use of amino acid reactive tags

114. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps: (a) making a modified cell by modifying the genetic composition of a cell; (b) culturing the modified cell to generate a plurality of modified cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifyng an engineered phenotype in the cell using real-time metabolic flux analysis.

115. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising addition of a nucleic acid to the cell.

116. The method of claim 115, wherein the nucleic acid comprises a nucleic acid heterologous to the cell.

117. The method of claim 115, wherein the nucleic acid comprises a nucleic acid homologous to the cell.

118. The method of claim 117, wherein the homologous nucleic acid comprises a modified homologous nucleic acid.

119. The method of claim 118, wherein the homologous nucleic acid comprises a modified homologous gene.

120. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell.

121. The method of claim 114, wherein the genetic composition of the cell is modified by a method comprising modifying or knocking out the expression of a gene.

122. The method of claim 114, further comprising selecting a cell comprising a newly engineered phenotype.

123. The method of claim 122, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

124. The method of claim 122, wherein the newly engineered phenotype is selected from the group consisting of an increased or decreased expression or amount of a polypeptide, an increased or decreased amount of an mRNA transcript, an increased or decreased expression of a gene, an increased or decreased resistance or sensitivity to a toxin, an increased or decreased resistance use or production of a metabolite, an increased or decreased uptake of a compound by the cell, an increased or decreased rate of metabolism, and an increased or decreased growth rate.

125. The method of claim 114, further comprising isolating a cell comprising a newly engineered phenotype.

126. The method of claim 114, wherein the newly engineered phenotype is a stable phenotype.

127. The method of claim 126, wherein modifying the genetic composition of a cell comprises insertion of a construct into the cell, wherein construct comprises a nucleic acid operably linked to a constitutively active promoter.

128. The method of claim 114, wherein the newly engineered phenotype is an inducible phenotype.

129. The method of claim 128, wherein modifying the genetic composition of a cell comprises insertion of a construct into the cell, wherein construct comprises a nucleic acid operably linked to an inducible promoter.

130. The method of claim 115, wherein nucleic acid added to the cell in step (a) is stably inserted into the genome of the cell.

131. The method of claim 115, wherein nucleic acid added to the cell in step (a) propagates as an episome in the cell.

132. The method of claim 115, wherein nucleic acid added to the cell in step (a) encodes a polypeptide.

133. The method of claim 132, wherein the polypeptide comprises a modified homologous polypeptide.

134. The method of claim 132, wherein the polypeptide comprises a heterologous polypeptide.

135. The method of claim 115, wherein the nucleic acid added to the cell in step (a) encodes a transcript comprising a sequence that is antisense to a homologous transcript.

136. The method of claim 114, wherein modifying the genetic composition of the cell in step (a) comprises increasing or decreasing the expression of an mRNA transcript.

137. The method of claim 114, wherein modifying the genetic composition of the cell in step (a) comprises increasing or decreasing the expression of a polypeptide.

138. The method of claim 114, wherein modifying the homologous gene in step (a) comprises knocking out expression of the homologous gene.

139. The method of claim 114, wherein modifying the homologous gene in step (a) comprises increasing the expression of the homologous gene.

140. The method of claim 114, wherein the heterologous gene in step (a) comprises a sequence-modified homologous gene, wherein the sequence modification is made by a method comprising the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises a homologous gene of the cell; (b) providing a plurality of oligonucleotides, wherein each oligonucleotide comprises a sequence homologous to the template polynucleotide, thereby targeting a specific sequence of the template polynucleotide, and a sequence that is a variant of the homologous gene; (c) generating progeny polynucleotides comprising non-stochastic sequence variations by replicating the template polynucleotide of step (a) with the oligonucleotides of step (b), thereby generating polynucleotides comprising homologous gene sequence variations.

141. The method of claim 114, wherein the heterologous gene in step (a) comprises a sequence-modified homologous gene, wherein the sequence modification is made by a method comprising the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises sequence encoding a homologous gene; (b) providing a plurality of building block polynucleotides, wherein the building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and a building block polynucleotide comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence; (c) combining a building block polynucleotide with a template polynucleotide such that the building block

polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations.

142. The method of claim 114, wherein the cell is a prokaryotic cell.

143. The method of claim 142, wherein the prokaryotic cell is a bacterial cell.

144. The method of claim 114, wherein the cell is a selected from the group consisting of a fungal cell, a yeast cell, a plant cell and an insect cell.

145. The method of claim 114, wherein the cell is a eukaryotic cell.

146. The method of claim 145, wherein the cell is a mammalian cell.

147. The method of claim 146, wherein the mammalian cell is a human cell.

148. The method of claim 114, wherein the measured metabolic parameter comprises rate of cell growth.

149. The method of claim 148, wherein the rate of cell growth is measured by a change in optical density of the culture.

150. The method of claim 114, wherein the measured metabolic parameter comprises a change in the expression of a polypeptide.

151. The method of claim 150, wherein the change in the expression of the polypeptide is measured by a method selected from the group consisting of a one-dimensional gel electrophoresis, a two-dimensional gel electrophoresis, a tandem mass spectography, an RIA, an ELISA, an immunoprecipitation and a Western blot.

152. The method of claim 114, wherein the measured metabolic parameter comprises a change in expression of at least one transcript, or, the expression of a transcript of a newly introduced gene.

153. The method of claim 152, wherein the change in expression of the transcript is measured by a method selected from the group consisting of a hybridization, a quantitative amplification and a Northern blot.

154. The method of claim 153, wherein transcript expression is measured by hybridization of a sample comprising transcripts of a cell or nucleic acid representative of or complementary to transcripts of a cell by hybridization to immobilized nucleic acids on an array.

155. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in a secondary metabolite.

156. The method of claim 155, wherein secondary metabolite is selected from the group consisting of a glycerol and a methanol.

157. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in an organic acid.

158. The method of claim 157, wherein the organic acid is selected from the group consisting of an acetate, a butyrate, a succinate and an oxaloacetate.

159. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in intracellular pH.

160. The method of claim 159, wherein the increase or a decrease in intracellular pH is measured by intracellular application of a dye, and the change in fluorescence of the dye is measured over time.

161. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in synthesis of DNA over time.

162. The method of claim 161, wherein the increase or a decrease in synthesis of DNA over time is measured by intracellular application of a dye, and the change in fluorescence of the dye is measured over time.

163. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in uptake of a composition.

164. The method of claim 163, wherein the composition is a metabolite.

165. The method of claim 164, wherein the metabolite is selected from the group consisting of a monosaccharide, a disaccharide, a polysaccharide, a lipid, a nucleic acid, an amino acid and a polypeptide.

166. The method of claim 165, wherein the saccharide, disaccharide or polysaccharide comprises a glucose or a sucrose.

167. The method of claim 163, wherein the composition is selected from the group consisting of an antibiotic, a metal, a steroid and an antibody.

168. The method of claim 114, wherein the measured metabolic parameter comprises an increase or a decrease in the secretion of a byproduct or a secreted composition of a cell.

169. The method of claim 168, wherein the byproduct or secreted composition is selected from the group consisting of a toxin, a lymphokine, a polysaccharide, a lipid, a nucleic acid, an amino acid, a polypeptide and an antibody.

170. The method of claim 114, wherein the real time monitoring simultaneously measures a plurality of metabolic parameters.

171. The method of claim 170, wherein real time monitoring of a plurality of metabolic parameters comprises use of a Cell Growth Monitor device.

172. The method of claim 171, wherein the Cell Growth Monitor device is a Wedgewood Technology, Inc., Cell Growth Monitor model 652.

173. The method of claim 171, wherein the real time simultaneous monitoring measures uptake of substrates, levels of intracellular organic acids and levels of intracellular amino acids.

174. The method of claim 171, wherein the real time simultaneous monitoring measures: uptake of glucose; levels of acetate, butyrate, succinate or oxaloacetate; and, levels of intracellular natural amino acids.

175. The method of claim 171, further comprising use of a computer-implemented program to real time monitor the change in measured metabolic parameters over time.

176. The method of claim 175, wherein the computer-implemented program comprises a computer-implemented method as set forth in FIG. 28.

177. The method of claim 176, wherein the computer-implemented method comprises metabolic network equations.

178. The method of claim 176, wherein the computer-implemented method comprises a pathway analysis.

179. The method of claim 176, wherein the computer-implemented program comprises a preprocessing unit to filter out the errors for the measurement before the metabolic flux analysis.

L15 ANSWER 2 OF 22 USPATFULL on STN
2005:143828 Lipoparticles comprising proteins, methods of making, and using the same.
Doranz, Benjamin J., Narberth, PA, UNITED STATES
Willis, Sharon, Wayne, PA, UNITED STATES
Ross, Eric, Philadelphia, PA, UNITED STATES
Greene, Tiffani Anne, Cherry Hill, NJ, UNITED STATES
US 2005123563 A1 20050609
APPLICATION: US 2004-901399 A1 20040728 (10)
PRIORITY: US 2003-491477P 20030730 (60)
US 2003-491633P 20030730 (60)
US 2003-498755P 20030829 (60)
US 2003-502478P 20030912 (60)
US 2003-509677P 20031007 (60)
US 2003-509608P 20031007 (60)
US 2003-509575P 20031007 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to lipoparticles. The invention also relates to producing lipoparticles. The invention further relates to

lipoparticles comprising a viral structural protein. The invention further relates to a lipoparticle comprising a membrane protein, and the lipoparticle can be attached to a sensor surface. The invention further relates to methods of producing and using the lipoparticle to, inter alia, assess protein binding interactions.

CLM What is claimed is:

1. A lipoparticle comprising a viral protein component and a cellular protein, wherein said viral protein component consists essentially of a viral structural protein.

2. A lipoparticle comprising a viral protein component and a cellular protein, wherein said cellular protein is an unmodified protein, and wherein said lipoparticle is reverse transcription incompetent.

3. The lipoparticle of claims 1 or 2 wherein said cellular protein comprises a multiple spanning membrane protein.

4. The lipoparticle of claims 1 or 2 wherein said cellular protein comprises a G protein coupled receptor.

5. The lipoparticle of claim 1, wherein said cellular protein is a non-membrane protein that has been modified to be capable of localizing to the membrane of a cell.

6. A lipoparticle comprising an unmodified viral structural protein and a cellular protein, provided that the only viral proteins in said lipoparticle are structural proteins.

7. The lipoparticle of claim 6, wherein the cellular protein is a multiple spanning membrane protein.

8. A lipoparticle comprising a viral structural protein and a native cellular protein, provided that the only viral proteins in said lipoparticle are structural proteins.

9. The lipoparticle of claim 8, wherein the cellular protein is a multiple-spanning membrane protein.

10. The lipoparticle of claims 1 or 6 wherein said viral structural protein is Gag.

11. The lipoparticle of claim 10 wherein said Gag is from murine leukemia virus or rous sarcoma virus.

12. A composition comprising an isolated lipoparticle of any of claims 1 or 6 attached to a biosensor surface.

13. A lipoparticle comprising a viral protein component, a cellular protein and a G-protein.

14. The lipoparticle of claim 13, wherein said G-protein is a modified G-protein.

15. The lipoparticle of claim 14, wherein said modified G-protein comprises a fusion protein.

16. The lipoparticle of claim 15, wherein said fusion protein comprises a fluorescent protein, a linker, a viral protein, membrane protein, a protease cleavage sequence, or combinations thereof.

17. The lipoparticle of claim 13, wherein said cellular protein is a GPCR.

18. The lipoparticle of claim 17 said GPCR is a modified GPCR.

19. The lipoparticle of claim 18, wherein said modified GPCR comprises a fusion protein.

20. The lipoparticle of claim 19, wherein said fusion protein comprises a fluorescent protein, a linker, a viral protein, membrane protein, a protease cleavage sequence, or combinations thereof.

21. The lipoparticle of claim 13 further comprising a GTP analog.

22. The lipoparticle of claim 21 wherein said GTP analog is a fluorescent GTP analog.

23. A method of identifying modulators of a GPCR comprising: a) contacting a lipoparticle comprising a GPCR and a G-protein with a test

compound; and b) measuring GPCR activity.

24. A method for producing a lipoparticle comprising: a) contacting a cell with nucleic acid encoding an unmodified viral structural protein and a cellular protein; and b) culturing said cell under conditions resulting in production of said lipoparticle, provided that the only viral protein encoded by said nucleic acid is a structural protein.

25. The method of claim 24 wherein a first nucleic acid molecule encodes said unmodified viral structural protein and a second nucleic acid molecule encodes said cellular protein.

26. The method of claim 24 wherein said contacting comprises infecting a cell with a non-enveloped virus, wherein said non-enveloped virus comprises a nucleic acid encoding the viral structural protein and a cellular protein.

27. The method of claim 26 wherein said non-enveloped virus is an adenovirus.

28. The method of claim 24 wherein said contacting comprises infecting a cell with an alphavirus, wherein said alphavirus comprises a nucleic acid encoding the viral structural protein and a cellular protein.

29. The method of claim 28, wherein said alphavirus is Semliki Forest Virus

30. The method of claim 24 wherein said cell is a non-human cell and said cellular protein is a human protein.

31. The method of claim 24 wherein the viral structural protein is Gag.

32. A method for producing a lipoparticle comprising: a) contacting a cell having a membrane protein of interest with an adenovirus encoding at least a viral Gag protein; and b) culturing said cell under conditions resulting in production of said lipoparticle.

33. A method for producing a lipoparticle comprising: a) contacting a cell with an adenoviral vector encoding at least a viral Gag protein and a cellular protein or an adenoviral vector encoding at least a viral Gag protein and a nucleic acid encoding said cellular protein; and b) culturing said cell under conditions resulting in production of said lipoparticle.

34. The method of any of claims 24, 32, or 33 wherein said protein is a membrane protein.

35. A **chimeric** viral vector comprising adenoviral nucleic acid and retroviral nucleic acid, provided that said retroviral nucleic acid comprises a sequence encoding Gag, but does not comprise a sequence encoding the envelope, promoter, or packaging signal of the retrovirus.

36. A method of eliciting an immune response in a subject comprising administering the composition of claims 1 or 6 to said subject.

37. A method of assessing the binding interaction of a protein with a ligand, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate, with a ligand of said protein; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said ligand; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said ligand assesses said binding interaction of said protein with said ligand.

38. A method of identifying potential ligands of a protein, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate, with a test ligand and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said ligand; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said ligand identifies a ligand.

39. A method of identifying a compound that affects binding between a ligand and a protein, said method comprising contacting said compound

with said ligand; contacting said compound/ligand complex with a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said compound is not contacted with said ligand and said ligand is contacted with said lipoparticle; wherein detecting a change in said substrate when said compound is contacted with said ligand compared with said otherwise identical substrate wherein said ligand is contacted with said lipoparticle assesses said effect of said compound.

40. A method of detecting a ligand of a protein in a test sample, said method comprising contacting a lipoparticle according to any of claims 1 or 6 comprising said protein, wherein said lipoparticle is attached to a substrate with a test sample; and detecting any change in said substrate compared with any change in an otherwise identical substrate wherein said lipoparticle is not contacted with said test sample; wherein detecting a change in said substrate wherein said lipoparticle is contacted with said ligand compared with said otherwise identical substrate wherein said lipoparticle is not contacted with said test sample indicates the presence of said ligand in said test sample.

41. The method of claim 24, wherein said nucleic acid comprise an adenovirus, baculovirus, vaccinia virus, herpes virus, or alphavirus vector.

42. An immunogen comprising a lipoparticle according to any of claims 1 or 6.

43. A method of eliciting an immune response to a protein, said method comprising the introduction of a lipoparticle to an animal, and said lipoparticle being produced using the method of any of the claims 24, 32, or 33.

44. A method of eliciting an immune response to a protein, said method comprising the introduction of a lipoparticle to an animal, and said lipoparticle being a lipoparticle according to any of claims 1 or 6.

45. A method of determining the structure of a membrane protein comprising: a) isolating a membrane protein from a lipoparticle containing said membrane protein; and b) determining said structure of said membrane protein; wherein said membrane protein is not a viral envelope protein.

46. A method of determining the structure of a protein comprising: a) isolating a protein from a lipoparticle containing said membrane protein; and b) determining said structure of said protein; wherein said protein comprises a Gag fusion protein.

47. A composition comprising an array of lipoparticles attached to a surface.

48. The composition of claim 47, wherein said surface comprises an optical biosensor surface, an optical fiber, a microfluidic device, a microcantilever, an acoustive wave sensor, or electromagnetic sensor.

49. The composition of claim 47, wherein said surface measures surface plasmon resonance, colorimetric diffraction grating, chemiluminescence, deflection of light, acoustic waves, or fluorescence.

50. The composition of claim 47, wherein said lipoparticles comprise membrane proteins derived from a naturally occurring source.

51. The composition of claim 50, wherein said naturally occurring source is a primary cell, an organ, a stem cell, or a cell line.

52. The composition of claim 47 wherein said surface has been modified to bind lipoparticles.

53. The composition of claim 52 wherein said modification comprises a cationic film, alkane modification, gamma-aminopropylsilane, histidine, Ni+2, protein A, protein G, protein L, lectin, biotin, or avidin.

54. The composition of claim 47 wherein said lipoparticles have been modified to bind to the surface.

55. A method of detecting an infectious pathogen in a sample comprising the steps of: a) contacting the sample with an array of lipoparticles attached to a surface, wherein said array of lipoparticles comprises

membrane proteins that interact with various infectious pathogens; and b) detecting an interaction with said array of lipoparticles; wherein said detection of said interaction indicates the presence of an infectious pathogen.

56. A method of determining the presence of a substance in a sample comprising the steps of: a) contacting the sample with an array of lipoparticles attached to a surface, wherein said lipoparticles comprise membrane proteins that interact with said substance; and b) detecting an interaction with said array of lipoparticles; wherein said detection of said interaction indicates the presence of said substance.

57. A method of identifying an inhibitor of a binding activity of a substance to a membrane protein comprising the steps of: a. contacting said substance with an array of lipoparticles comprising said membrane protein attached to a surface to which said substance normally binds, in the presence of a potential inhibitor; and b. detecting an interaction of said substance with said array; wherein if an interaction is detected, then said potential inhibitor does not inhibit said binding and if an interaction is not detected then said potential inhibitor inhibits said binding.

58. The method of claim 57, wherein said potential inhibitor comprises a fluorescent tag, enzymatic tag, biotinylated tag, paramagnetic tag, a radioactive tag, or a combination thereof.

59. The method of any of claims 56 or 57, wherein said substance is an organic compound, chemical, peptide, protein, antibody, virus, bacteria, toxin, a monoclonal antibody, a low-molecular weight organic compound or a combination thereof.

60. A method for spotting lipoparticles, viruses, or virus-like particles in an array format onto a surface comprising including in the spotting medium a preservative.

61. The method of claim 60, wherein said preservative is trehalose, glycerol, collagen, sucrose, gelatin, or combinations thereof.

62. A method of identifying a binding partner of a membrane protein comprising: a) contacting a surface coated with lipoparticles, viruses, or virus-like particles comprising said membrane protein with an array comprising potential binding partners; and b) detecting binding of potential binding partner to said membrane protein.

63. The method of claim 62 wherein said potential binding partners comprise an antibody, antibody fragment, peptide, polypeptide, or small organic molecule.

64. A lipoparticle comprising a viral protein component and a cellular protein, wherein said viral protein component consists essentially of a viral structural protein, wherein said cellular protein is an ion channel protein or transporter protein.

65. The lipoparticle of claim 64 wherein said viral structural protein is unmodified.

66. The lipoparticle of claim 64, wherein said lipoparticle comprises a fluorescent dye.

67. The lipoparticle of claim 64, wherein said lipoparticle comprises at least one modification to detect ion channel protein function or transporter protein function

68. A method to determine membrane protein function in a lipoparticle, virus, or virus-like particle comprising a membrane protein, wherein said lipoparticle, virus, or virus-like particle further comprises a detectable agent, wherein measuring either an increase or decrease in the detectable agent is used to determine the membrane protein function.

69. The method of claim 68, wherein said membrane protein is an ion channel.

70. The method of claim 68, wherein said detectable agent is a fluorescent probe.

71. The method of claim 70, wherein said fluorescent probe is a voltage-sensitive fluorescent dye, an ion-sensitive fluorescent dye, fluorescent lipid, fluorescent amino acid, fluorescent nucleic acid, or a fluorescent protein.

72. The method of claim 68, wherein said increase or decrease in detectable signal indicates an activation of said protein.

73. The method of claim 68, wherein said detectable agent comprises at least two fluorescent probes that interact by resonance energy transfer to produce a distinct fluorescent signal, wherein said distinct signal is used to determine said function of said protein.

74. A method of identifying a stimulator of a membrane protein comprising: a) contacting a lipoparticle comprising said membrane protein and a detectable agent with a compound; and b) measuring any change in the detectable agent; wherein said change in the detectable agent is used to indicate that said compound is a stimulator.

75. A method of identifying an inhibitor of a known stimulator of an ion channel protein or a transporter protein within a lipoparticle, wherein said lipoparticle comprises an ion channel or transporter, comprising the steps of: a) contacting said lipoparticle with said stimulator; b) contacting said lipoparticle with a test compound; c) measuring the function of said ion channel protein or transporter protein.

76. A method of detecting changes in ion concentration in a location comprising: a) microinjecting lipoparticles comprising a membrane protein and a detectable agent to said location; and b) detecting changes in ion concentration by measuring said change in said detectable agent.

77. An immunogenic composition comprising a lipoparticle comprising a protein of interest and at least one immunostimulatory component.

78. The immunogenic composition of claim 77, wherein said at least one immunostimulatory component comprises DNA, adenovirus, adjuvant, or proteins.

79. A method of producing antibodies against a protein comprising: a) administering the immunogenic composition according to claim 77 comprising said protein to an animal; and b) isolating said antibodies.

80. A method of identifying a binding partner of a membrane protein comprising: a) contacting a lipoparticle, virus, or virus-like particle comprising said membrane protein with a library, wherein said library comprises more than one potential binding partner; b) detecting the binding of said binding partner to said membrane protein.

81. The method of claim 80 wherein said library is a phage display library or ribosome display library.

82. The method of claim 80 wherein said library comprises antibodies.

83. The method of claim 82 wherein said antibody is a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single-chain Fv, an Fc-fusion protein, peptide, or polypeptide.

84. A method of transfecting a protein into a cell comprising contacting said cell with a lipoparticle comprising said protein.

85. A method of transfecting a protein into a cell comprising contacting said cell with a lipoparticle comprising a viral protein component and said protein, wherein said viral protein component consists essentially of a viral structural protein.

86. A method of correcting a protein defect in an individual comprising administering a cell transfected according to any of claims 84 or 85.

87. A particle comprising a fluorophore wherein said fluorophore changes fluorescence in response to pH, membrane potential, oxidation state, NO level, ion concentration, ATP concentration, protein interaction, or combinations thereof m in size and wherein said particle is less than 1 μm.

88. The particle of claim 87, wherein said particle is a lipoparticle.

89. The particle of claim 87, wherein said particle is a virus particle or virus-like particle.

90. The particle of claim 87, wherein said fluorophore is a fluorescent

protein or fluorescent dye.

91. The particle of claim 90 wherein said fluorescent protein comprises a fusion protein comprising Gag.

92. The particle of claim 90 wherein said fluorescent protein is a membrane protein or membrane protein fusion protein.

93. A lipoparticle comprising a Gag fusion protein and exogenous membrane protein, wherein said Gag fusion protein comprises a fluorescent protein or an enzymatic protein.

94. The lipoparticle of claim 93 wherein said membrane protein comprises a binding domain.

95. The lipoparticle of claim 94 wherein said binding domain comprises the ligand-binding portion of a cellular membrane protein.

96. The lipoparticle of claim 94 wherein said binding domain comprises an antibody-binding domain.

97. The lipoparticle of claim 96 wherein said antibody-binding domain is from Protein A, Protein G, or Protein L.

98. The lipoparticle of claim 97 further comprising one or more antibody-like molecules bound to said binding domain.

99. The lipoparticle of claim 98 wherein said antibody-like molecules comprises a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single chain Fv, an Fc-fusion protein, or combinations thereof.

100. The lipoparticle of claim 93 wherein said exogenous membrane protein comprises a reporter fusion protein.

101. A lipoparticle comprising a modified lipid.

102. The lipoparticle of claim 101 wherein said modified lipid comprises a biotin-coupled or a fluorescently-coupled lipid.

103. A lipoparticle comprising at least one of a radioactive molecule, a magnetic substance, a paramagnetic substance, a biotinylated molecule, an avidin-like molecule, gold, or combinations thereof and optionally a fluorophore.

104. A method of incorporating a molecule into a lipoparticle, virus or a virus-like particle comprising contacting an AM-ester form of said molecule with a lipoparticle comprising an esterase.

105. A method of incorporating a molecule into a lipoparticle, virus or a virus-like particle comprising contacting a soluble form of said molecule with said lipoparticle and performing electroporation, sonication, or vortexing.

106. A method of inducing pores in a lipoparticle comprising incubating said lipoparticle with a pore-forming peptide, an alkane, or a detergent.

107. A method of attaching a molecule to a lipoparticle, virus, or virus-like particle comprising contacting a modified molecule with said lipoparticle, virus, or virus-like particle, wherein said lipoparticle, virus, or virus-like particle is able to bind to said modified molecule.

108. The method of claim 107 wherein said modified molecule comprises a biotin group or avidin group.

109. The method of claim 107 wherein said modified molecule comprises a lectin binding molecule.

110. A method of determining binding of a compound to a lipoparticle, virus, or virus-like particle comprising a) contacting said compound with said lipoparticle; and b) determining if said compound binds to said lipoparticle, wherein said compound or said compound and said lipoparticle comprises a fluorescent label.

111. The method of claim 110 wherein said determining comprises using a microscope, VELISA, flow cytometry, AVELISA, or immunofluorescence.

112. The method of claim 110 wherein said lipoparticle is attached to a bead.

113. The method of claim 110, wherein said compound is a ligand, a peptide, a protein, an antibody, an organic chemical compound, or an inorganic chemical compound.

114. A method of detecting the presence of an antigen in a sample comprising: a) contacting a lipoparticle comprising a binding partner for said antigen with said sample; and b) detecting a signal in said sample; wherein said detection of said signal indicates the presence of said antigen.

115. The method of claim 114 wherein said lipoparticle comprises an antibody-binding domain.

116. The method of claim 114 wherein said method is used to identify a pathogen, a disease diagnosis, or a drug.

117. A method of hybridizing an oligonucleotide to a target sequence in a lipoparticle, virus, or virus-like particle comprising contacting said oligonucleotide with said lipoparticle, virus, or virus-like particle comprising said target sequence under conditions that permit hybridization of said oligonucleotide to said target sequence.

118. The method of claim 117 wherein said target sequence is specific for a virus or virus family.

119. A method of detecting lipoparticle fusion comprising: a) contacting a lipoparticle, virus, or virus-like particle containing a fusigenic membrane protein with a lipoparticle comprising a receptor for said fusigenic membrane protein; and b) detecting said fusion; wherein said lipoparticle, virus, or virus-like particle comprises at least one reporter that is detectable upon fusion.

120. The method of claim 119 wherein said detection comprises detection by FRET, enzymatic activity, AM-ester cleavage, quenching, dequenching, or pyrene eximer formation.

121. A lipoparticle, virus, or virus-like particle attached to a bead, wherein said lipoparticle is attached to said bead via WGA, PEI, avidin-biotin interaction, poly-lysine interaction, or covalent coupling.

122. The lipoparticle of claim 121 wherein said lipoparticle comprises a first fluorescent label and said bead comprises a second fluorescent label.

123. A method for calculating the number of lipoparticles, viruses, or virus-like particles in a sample comprising: a) labeling said particles with a fluorophore; b) detecting said labeled particles, and c) counting said particles.

124. A method for calculating the quantity of particles, wherein said particles are lipoparticles, viruses, or virus-like particles comprising: a) measuring a detectable properties of a particle sample; and b) determining said quantity of particles by a correlation of amount of said properties to an amount of said particles.

125. A method for detecting the structural integrity of a membrane protein within a particle comprising a) contacting said particle with a molecule that binds to said membrane protein; and b) detecting binding of said molecule to said particle; wherein binding of said molecule to said particle is indicative that the structural integrity of said membrane protein is intact.

126. The method of claim 125 wherein said molecule is an antibody, conformation-dependent antibody, ligand, agonist, toxin, or antagonist.

127. The method of claim 125 wherein said membrane protein is an ion channel, transporter protein, or a GPCR.

128. A method for determining the purity of a particle, wherein said particle is a lipoparticle, virus, or virus-like particle preparation comprising: a) quantifying number of particles in said preparation; b) quantifying total protein concentration in said preparation; and c) determining said purity by dividing the total protein concentration by the number of particles; and d) dividing the number obtained from step c) by the theoretical protein weight of said particle, wherein a value

of about 1 is indicative of a pure sample and a value greater than 1 is indicative of a sample that is not completely pure.

129. A lipoparticle comprising at least one fusion protein, wherein said fusion protein comprises at least one binding domain, at least one transmembrane domain, and at least one reporter domain.

130. The lipoparticle of claim 129, wherein said binding domain comprises an antibody-binding domain.

131. The lipoparticle of claim 129 wherein said antibody-binding domain is an antibody-binding domain from Protein A, Protein G, or Protein L.

132. The lipoparticle of claim 129 wherein said binding domain comprises a ligand-binding portion of a cellular membrane protein

133. The lipoparticle of claim 129 wherein said reporter domain comprises a fluorescent protein.

134. The lipoparticle of claim 129 wherein said reporter domain comprises an amino acid sequence comprising enzymatic activity.

135. The lipoparticle of claim 129 wherein said reporter domain comprises an amino acid sequence comprising an inactive form of a protein that is made functional when brought into close proximity with a complementary inactive form.

136. The lipoparticle of claim 135, wherein said reporter comprises a fluorescent protein or an enzyme.

137. The lipoparticle of claim 129, wherein said lipoparticle comprises a first fusion protein and a second fusion protein, wherein each fusion protein comprises at least one binding domain, at least one transmembrane domain, and at least one reporter domain.

138. The lipoparticle of claim 137, wherein said first and second fusion proteins comprise two different reporter domains.

139. The lipoparticle of claim 137, wherein each of said first and second fusion proteins comprise an antibody-binding domain and wherein said lipoparticle further comprises one or more antibody-like molecules bound to said antibody-binding domain.

140. The lipoparticle of claim 139, wherein each of said antibody-like molecules comprises a monoclonal antibody, a polyclonal antibody, an affinity-purified polyclonal antibody, a Fab fragment derived from a monoclonal antibody, an immunoglobulin-fusion protein, a single-chain Fv, an Fc-fusion protein, or combinations thereof.

141. The lipoparticle of claim 139, wherein said first and second fusion proteins comprise two different antibody-like molecules that each recognizes different epitopes on the same protein.

142. The lipoparticle of claim 139, wherein said first and second fusion proteins comprise two different antibody-like molecules that each recognizes different epitopes on different proteins.

143. A method of detecting the presence of an antigen in a sample comprising contacting said sample with at least one lipoparticle comprising a binding partner wherein said particle comprises at least one fusion protein comprising at least one binding domain, at least one transmembrane domain, and at least one reporter domain and detecting the signal from said lipoparticle.

144. The method of claim 143 wherein said sample comprises at least one pathogenic protein, at least one whole-pathogenic organism, at least one secreted cellular peptide, at least one immobilized protein, at least one tissue section, at least one cell, at least one antibody, or combinations thereof.

145. The method of claim 143, wherein said at least one lipoparticle comprises an array of lipoparticles.

146. The method of claim 143, wherein said antigen comprises an array of antigens.

147. A device comprising at least one lipoparticle and capable of being used to perform the method of claim 143.

L15 ANSWER 3 OF 22 USPATFULL on STN
2005:117278 **Multivalent** carriers of bi-specific antibodies.
Hansen, Hans J., Picayune, MS, UNITED STATES
McBride, William J., Boonton, NJ, UNITED STATES
Qu, Zhengxing, Warren, NJ, UNITED STATES
Immunomedics, Inc., Morris Plains, NJ, UNITED STATES (U.S. corporation)
US 2005100543 A1 20050512
APPLICATION: US 2004-882151 A1 20040701 (10)
PRIORITY: US 2003-483832P 20030701 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Provided herein are targetable constructs that are **multivalent** carriers of bi-specific antibodies, i.e., each molecule of a targetable construct can serve as a carrier of two or more bi-specific antibodies. Also provided are targetable complexes formed by the association of a targetable construct with two or more bi-specific antibodies. The targetable constructs and targetable complexes of the invention are incorporated into biosensors, kits and pharmaceutical compositions, and are used in a variety of therapeutic and other methods.

CLM What is claimed is:

1. A bi-specific antibody comprising the structure $[IgG_1]$-[scFv]2; wherein said antibody comprises a pair of heavy chains and a pair of light chains, wherein each heavy chain comprises an IgG1 heavy chain and an scFv, wherein said scFv is fused to the C-terminus of said IgG 1 heavy chain, optionally via a linker peptide.

2. The antibody according to claim 1, wherein the binding sites formed by said heavy chain and said light chain specifically binds to an epitope on a targeted tissue.

3. The antibody according to claim 2, wherein each of said scFv moieties specifically binds to a carrier epitope.

4. The antibody according to claim 1, wherein said IgG1 is a human, humanized, **chimeric**, or CDR-grafted antibody.

5. The antibody according to claim 1, wherein each of said scFv molecules is human, humanized, or CDR-grafted.

6. The antibody according to claim 5, wherein said antibody further comprises a bioactive moiety.

7. The antibody according to claim 6, wherein said bioactive moiety is selected from the group consisting of a drug, a prodrug, an enzyme, a hormone, an immunomodulator, an oligonucleotide; a radionuclide, an image enhancing agent and a toxin.

8. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hMN14-IgG1]-$[734scFv]_2$ and [hMN14-IgG1(1253A)]-$[734scFv]_2$.

9. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hMN14-IgG1]-$[679scFv]_2$ and [hMN14-IgG1(1253A)]-$[679scFv]_2$.

10. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hA20-IgG1]-$[734scFv]_2$ and [hA20-IgG1(1253A)]-$[734scFv]_2$.

11. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hA20-IgG1]-$[679scFv]_2$ and [hA20-IgG1(1253A)]-$[679scFv]_2$.

12. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hLL2-IgG1]-$[734scFv]_2$ and [hLL2-IgG1(1253A)]-$[734scFv]_2$.

13. The antibody according to claim 1, wherein said antibody is selected from the group consisting of [hLL2-IgG1]-$[679scFv]_2$ and [hLL2-IgG1(1253A)]-$[670scFv]_2$.

14. A binding complex comprising a tetravalent binding molecule bound to a targetable construct, wherein said tetravalent binding molecule comprises two binding sites for a carrier epitope and two binding sites for a target epitope, and wherein said targetable construct comprises a molecular scaffold and at least two carrier epitopes.

15. The binding complex according to claim 14, wherein said targetable construct comprises at least two pairs of carrier epitopes and wherein at least two of said tetravalent binding molecules are bound to said targetable construct.

16. The binding complex according to claim 15, wherein said at least two pairs of carrier epitopes comprise a first pair and a second pair, wherein said first and second pair are different epitopes, and wherein a first tetravalent binding molecule is bound to said first pair of carrier epitopes and a second tetravalent binding molecule is bound to said second pair of carrier epitopes.

17. The binding complex according to claim 16, wherein said first and second pair of carrier epitopes are different epitopes.

18. The binding complex according to claim 17, wherein said first and second tetravalent binding molecules bind to the same target epitope.

19. The binding complex according to claim 14, wherein said targetable construct is selected from the group consisting of IMP 246, IMP 156, IMP 192 and IMP 222.

20. The binding complex according to claim 14, wherein said carrier epitope is a hapten.

21. The binding complex according to claim 14, wherein said carrier epitope is a chelator, wherein said chelator optionally is bound to a metal ion.

22. The binding complex according to claim 21, wherein said chelator is selected from the group consisting of DTPA, DOTA, benzyl DTPA, NOTA, and TETA.

23. The binding complex according to claim 14, wherein said tetravalent binding molecule is a bi-specific antibody comprising the structure $[IgG_1]$-[scFv]2; wherein said antibody comprises a pair of heavy chains and a pair of light chains, wherein each heavy chain comprises an IgG1 heavy chain and an scFv, wherein said scFv is fused to the C-terminus of said IgG1 heavy chain, optionally via a linker peptide.

24. The binding complex according to claim 14, wherein said molecular scaffold is a peptide or peptide derivative.

25. The binding complex according to claim 14, wherein said target epitope is an antigen associated with a disease.

26. The binding complex according to claim 25, wherein said disease is selected from the group consisting of hyperproliferative disease, pathogenic disease, cancer, cardiovascular disease, neurodegenerative disease, metabolic disease, and autoimmune disease

27. The binding complex according to claim 26, wherein said target epitope is a tumor associated antigen associated with a type of cancer selected from the group consisting of acute lymphoblastic leukemia, acute myelogenous leukemia, biliary cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal cancer, endometrial cancer, esophageal, gastric, head and neck cancer, Hodgkin's lymphoma, lung cancer, medullary thyroid, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, glioma, melanoma, liver cancer, prostate cancer, and urinary bladder cancer.

28. The binding complex according to claim 27, wherein said target epitope is a tumor associated antigen selected from the group consisting of A3, antigen specific for A33 antibody, BrE3, CD1, CD1a, CD3, CD5, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD45, CD74, CD79a, CD80, HLA-DR, NCA 95, NCA90, HCG and its subunits, CEA, CSAp, EGFR, EGP-1, EGP-2, Ep-CAM, Ba 733, HER2/neu, KC4, KS-1, KS1-4, Le-Y, MAGE, MUC1, MUC2, MUC3, MUC4, PAM-4, PSA, PSMA, RS5, S100, TAG-72, p53, tenascin, IL-6, insulin growth factor-1 (IGF-1), Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, 17-1A, an angiogenesis marker, a cytokine, an immunomodulator, an oncogene marker, an oncogene product, and other tumor associated antigens.

29. A method of treating a disease in a subject, comprising administering to a subject suffering from said disease (i) a tetravalent binding molecule comprising two binding sites for a carrier epitope and two binding sites for a target epitope, wherein said target epitope is an epitope associated with said disease, (ii) optionally, a clearing agent, and (iii) a targetable construct comprising a molecular

scaffold and at least two carrier epitopes.

30. The method according to claim 29, wherein said disease is selected from the group consisting of hyperproliferative disease, pathogenic disease, cancer, cardiovascular disease, neurodegenerative disease, metabolic disease, and autoimmune disease.

31. The method according to claim 29, wherein said targetable construct further comprises a bioactive moiety.

32. A method of diagnosing/detecting a disease in a subject, comprising administering to a subject suspected of suffering from said disease (i) a tetravalent binding molecule comprising two binding sites for a carrier epitope and two binding sites for a target epitope, (ii) optionally, a clearing agent, and (iii) a targetable construct comprising a molecular scaffold and at least two carrier epitopes, wherein said construct comprises a detectable label.

33. The method according to claim 32, wherein said target epitope is comprised within, displayed by or released from one or more cells, tissues, organs or systems of said subject.

34. A kit, comprising (i) a tetravalent binding molecule comprising two binding sites for a carrier epitope and two binding sites for a target epitope, (ii) optionally, a clearing agent, and (iii) a targetable construct comprising a molecular scaffold and at least two carrier epitopes.

35. A pharmaceutical composition comprising a bispecific antibody according to claim 1.

L15 ANSWER 4 OF 22 USPATFULL on STN
2005:36953 **Chimeric** antigens for breaking host tolerance to foreign antigens.
George, Rajan, Edmonton, CANADA
Tyrrell, Lorne, Edmonton, CANADA
Noujaim, Antoine, Edmonton, CANADA
Wang, Dakun, Edmonton, CANADA
Ma, Allan, Edmonton, CANADA
ViRexx Medical Corp., Edmonton, CANADA (non-U.S. corporation)
US 2005031628 A1 20050210
APPLICATION: US 2004-913171 A1 20040805 (10)
PRIORITY: US 2003-493449P 20030808 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed herein are compositions and methods for eliciting immune responses against antigens. In particular, the compounds and methods elicit immune responses against foreign antigens that are otherwise recognized by the host as "self" antigens, thus breaking host tolerance to those antigens. Presenting the host immune system with a **chimeric** antigen comprising an immune response domain and a target binding domain, wherein the target binding domain comprises an antibody fragment, enhances the immune response against the foreign or tolerated antigen. Antigen presenting cells take up, process, and present the **chimeric** antigen, eliciting both a humoral and cellular immune response against the desired antigen.
CLM What is claimed is:
1. A **chimeric** antigen for eliciting an immune response comprising an immune response domain and a target binding domain, wherein the target binding domain comprises an antibody fragment.

2. The **chimeric** antigen of claim 1, wherein the antibody fragment is not a xenotypic antibody fragment.

3. The **chimeric** antigen of claim 1, wherein the **chimeric** antigen comprises more than one immune response domain.

4. The **chimeric** antigen of claim 1, wherein the immune response domain comprises at least one antigenic portion of a protein selected from the group consisting a HBV protein, a DHBV protein, a HCV protein, a HPV protein, a HIV protein, a HSV protein, protein from an obligate intracellular parasite or a cancer antigen.

5. The **chimeric** antigen of claim 4, wherein the immune response domain comprises at least one antigenic portion of a HBV protein selected from the group consisting of HBV S1/S2, HBV S1/S2/S, HBV Core, HBV Core ctm and HBV polymerase.

6. The **chimeric** antigen of claim 4, wherein the immune response domain

comprises at least one antigenic portion of a DHBV protein selected from the group consisting of DHBV PreS/S, DHBV PreS, DHBV Core and DHBV polymerase.

7. The **chimeric** antigen of claim 4, wherein the immune response domain comprises at least one antigenic portion of a HCV protein selected from the group consisting of HCV Core (1-191), HCV Core (1-177), HCV E1-E2, HCV E1, HCV E2, HCV NS3, HCV NS5A and HCV NS4A.

8. The **chimeric** antigen of claim 1, wherein the target binding domain is capable of binding to an antigen presenting cell.

9. The **chimeric** antigen of claim 1, wherein the antibody fragment comprises an immunoglobulin heavy chain fragment.

10. The **chimeric** antigen of claim 9, wherein the immunoglobulin heavy chain fragment comprises a hinge region.

11. The **chimeric** antigen of claim 1, wherein the antibody fragment is a Fc fragment.

12. The **chimeric** antigen of claim 1, further comprising a linker for linking the immune response domain and the target binding domain.

13. The **chimeric** antigen of claim 12, wherein the linker is a covalent peptide linkage.

14. The **chimeric** antigen of claim 13, wherein the peptide linkage comprises the sequence SRPQGGGS or VRPQGGGS (SEQ ID NO: 1).

15. The **chimeric** antigen of claim 12, wherein the linker is selected from the group consisting of leucine zippers and biotin/avidin.

16. The **chimeric** antigen of claim 1, wherein the **chimeric** antigen is mannose glycosylated.

17. The **chimeric** antigen of claim 1, which elicits a multi-epitopic response.

18. The **chimeric** antigen of claim 1, which elicits an immune response to at least one epitope of the immune response domain.

19. The **chimeric** antigen of claim 1, which elicits a humoral immune response.

20. The **chimeric** antigen of claim 1, which elicits a cellular immune response.

21. The **chimeric** antigen of claim 1, which elicits a Th1 immune response, a Th2 immune response, a CTL response, or a combination thereof.

22. A method of enhancing antigen presentation in an antigen presenting cell comprising contacting the antigen presenting cell with a composition comprising a **chimeric** antigen of claim 1.

23. The method of claim 22, wherein the antigen presenting cell is a dendritic cell.

24. The method of claim 22, wherein the method enhances antigen presentation of more than one epitope.

25. The method of claim 22, wherein the method enhances antigen presentation of at least one epitope of the immune response domain.

26. The method of claim 22, wherein the contacting takes place ex vivo.

27. The method of claim 22, wherein the contacting takes places in vivo.

28. The method of claim 27, wherein the contacting takes place in a human.

29. A method of activating an antigen presenting cell comprising contacting the antigen presenting cell with a **chimeric** antigen of claim 1.

30. The method of claim 29, wherein the contacting takes place ex vivo.

31. The method of claim 29, wherein the contacting takes places in vivo.

32. The method of claim 31, wherein the contacting takes place in a human.

33. A method of eliciting an immune response comprising administering to a subject a composition comprising a **chimeric** antigen of claim 1.

34. The method of claim 33, wherein the subject has an infection or a cancer.

35. The method of claim 34, wherein the infection is a viral infection or a parasitic infection.

36. The method of claim 35, wherein the infection is a chronic viral infection.

37. A method of breaking tolerance comprising contacting an antigen presenting cell with a **chimeric** antigen of claim 1.

38. The method of claim 37, wherein administration of the immune response domain alone does not elicit an immune response.

39. The method of claim 37, wherein administration of the **chimeric** antigen elicits a greater immune response than administration of the immune response domain alone.

40. The method of claim 37, wherein the subject has an infection or a cancer.

41. The method of claim 40, wherein the infection is a viral infection or a parasitic infection.

42. The method of claim 41, wherein the infection is a chronic viral infection.

43. A method for treating an immune-treatable condition comprising administering, to a subject in need thereof, a **chimeric** antigen of claim 1.

44. The method according of claim 43, wherein the immune-treatable condition is an infection or a cancer.

45. The method of claim 44, wherein the infection is a viral infection or a parasitic infection.

46. The method of claim 45, wherein the infection is a chronic viral infection.

47. The method of claim 46, wherein the chronic viral infection is a chronic hepatitis B viral infection or a chronic hepatitis C viral infection, a chronic human papilloma viral infection, a chronic human immunodeficiency viral infection, or a chronic herpes simplex viral infection.

48. The method of claim 46, wherein the infection is a hepatitis B viral infection and the immune response domain comprises at least one antigenic portion of a protein selected from the group consisting of HBV Core protein, a HBV S protein, a HBV S1 protein and a HBV S2 protein and combinations thereof.

49. The method of claim 46, wherein the infection is a hepatitis C viral infection and the immune response domain comprises at least one antigenic portion of a protein selected from the group consisting of a HCV core (1-191) protein, a HCV Core (1-177) protein, a HCV E1 protein, a HCV E2 protein, a HCV E1-E2 protein, a HCV NS3 protein, a HCV NS5A protein and combinations thereof.

50. A method of vaccinating a subject against a viral infection comprising administering to the subject, a **chimeric** antigen of claim 1.

51. The method of claim 50, wherein the subject is therapeutically vaccinated against an existing viral infection.

52. The method of claim 50, wherein the subject is prophylactically vaccinated against a viral infection.

53. The method of claim 50, wherein the subject develops an immune response to more than one epitope of the **chimeric** antigen.

54. The method of claim 53, wherein the subject develops an immune response to more than one epitope of the immune response domain.

55. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a **chimeric** antigen of claim 1.

56. The pharmaceutical composition of claim 55, wherein the pharmaceutical composition is formulated for parenteral administration.

57. The pharmaceutical composition of claim 56, wherein the pharmaceutical composition is formulated for transdermal, intradermal, intravenous, subcutaneous, intramuscular, nasal, pulmonary or oral administration.

58. An article of manufacture comprising a **chimeric** antigen of claim 1 and instructions for administering the **chimeric** antigen to a subject in need thereof.

L15 ANSWER 5 OF 22 USPATFULL on STN
2004:306490 Methods and compositions for administering therapeutic and diagnostic agents.
McBride, William J., Boonton, NJ, UNITED STATES
Hansen, Hans J., Picayune, MS, UNITED STATES
Chang, Chien-Hsing Ken, Downington, PA, UNITED STATES
Goldenberg, David M., Mendham, NJ, UNITED STATES
Immunomedics, Inc. (U.S. corporation)
US 2004241158 A1 20041202
APPLICATION: US 2004-768707 A1 20040202 (10)
PRIORITY: US 2003-444357P 20030131 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and compositions are described for targeting therapeutic and diagnostic molecules to particular types of cells using targeting antibodies or other targeting moeities.
CLM What is claimed is:
1. A method for targeted delivery of a therapeutic or diagnostic agent, comprising administering to a mammal a primary targeting agent comprising at least one target binding moiety and at least one targetable construct binding moiety; a targetable construct comprising at least one primary targeting agent binding moiety, a clearing agent binding moiety, and a therapeutic or diagnostic moiety; and a clearing agent comprising at least one targetable construct binding moiety, wherein said clearing agent enhances retention of said targetable construct at a target site.

2. The method of claim 1, wherein said primary targeting agent binding moeity is orthogonal to said clearing agent binding moiety.

3. The method of claim 2, wherein said clearing agent comprises at least 2 targetable construct binding moieties.

4. The method of claim 3, wherein said primary targeting agent comprises a targetable construct binding antibody construct and a target binding antibody construct; said targetable construct comprises at least 2 copies of a hapten that bind with said targetable construct binding antibody construct, and a hapten that binds with a clearing agent antibody construct; and said clearing agent comprises a targetable construct binding antibody construct that binds with at least 2 targetable constructs.

5. The method of claim 4, wherein a targetable construct binding antibody of said clearing agent compri'ses an IgG antibody.

6. The method of claim 5, wherein said IgG antibody comprises an IgGI antibody.

7. The method of claim 4, wherein a targetable construct binding antibody of said clearing agent comprises an IgG antibody with a deleted C_{H2} domain that clears from circulation more rapidly than a corresponding IgG antibody that does not have said C_{H2} domain deleted.

8. The method of claim 4, wherein said targetable construct binding antibody construct of said clearing agent further comprises galactose.

9. The method of claim 1, wherein said clearing agent binds with a plurality of targetable constructs.

10. The method of claim 1, wherein binding of clearing agent to targetable construct stabilizes binding of targetable construct to primary targeting agent at binding sites and enhances clearance of targetable construct not bound to primary targeting agent at binding sites.

11. The method of claim 1, wherein said primary targeting agent and said clearing agent comprise at least one humanized antibody, **chimeric** antibody, human antibody, murine antibody or an antigen binding fragment thereof.

12. The method of claim 1, wherein said primary targeting agent further comprises at least one therapeutic moiety or at least one diagnostic moiety.

13. The method of claim 1 or 12 , wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activate a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

14. The method of claim 13, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, $^{123124}I$, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

15. The method of claim 1 or 12, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tci, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

16. The method of claim 1, wherein the primary targeting agent binds to at least one of the antigens selected from the group consisting of carcinoembryonic antigen (CEA), colon-specific antigen-p (CSAp), CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33,

CD37, CD38, CD40, CD40L, CD45, CD46, CD52, CD66a-d, CD74, CD75, CD80, CD126, B7, HLA-DR, Ia, Ii, HM1.24, MUC 1, MUC 2, MUC 3, MUC 4, NCA, EGFR, HER 2/neu, PAM-4, TAG-72, EGP-1, EGP-2, AFP, HCG, HCG-beta, PLAP, PAP, histone, A3, KS-1, Le(y), S100, PSMA, PSA, tenascin, folate receptor, VEGF, PlGF, ILGF-1 (insulin-like growth factor-1), necrosis antigens, IL-2, IL-6, T101, MAGE, organotropic hormones, oncogene products, cytokeratin, gangliosides and combinations thereof.

17. The method of claim 1, wherein the mammal has a disease or condition selected from the group consisting of cancer, autoimmune disease, infectious disease, a pathological disease associated with amyloid protein accumulation and cardiovascular lesions.

18. The method of claim 1, wherein said primary targeting agent and said targetable construct are administered simultaneously, and said clearing agent is administered subsequently to said simultaneous administration or at the same time.

19. A method for enhancing cellular internalization of a therapeutic or diagnostic agent, comprising administering to a mammal a primary targeting agent comprising at least one target binding moiety; and a separate internalization agent comprising an internalization moiety, wherein said primary targeting agent forms a complex with said internalization agent thereby enhancing internalization; and said complex further comprises a therapeutic or diagnostic moiety.

20. The method of claim 19, wherein said primary targeting agent further comprises a therapeutic or diagnostic moiety.

21. The method of claim 19, further comprising administering to said mammal a targetable construct comprising a primary targeting agent binding moiety, and a clearing agent binding moiety; and a clearing agent comprising a targetable construct binding moiety and an internalization moiety.

22. The method of claim 19, further comprising administering to said mammal a targetable construct comprising a primary targeting agent binding moiety, an internalization agent binding moiety and a clearing agent binding moiety; a clearing agent comprising a targetable construct binding moiety; and an internalization agent comprising a targetable construct binding moiety and an internalization moiety.

23. The method of claim 19 or 21, wherein said primary targeting agent and said clearing agent comprise at least one humanized antibody, **chimeric** antibody, human antibody, murine antibody or an antigen binding fragment thereof.

24. The method of claim 19, wherein said internalization moiety comprises a moiety bound by a folate receptor.

25. The method of claim 24, wherein said moiety bound by a folate receptor comprises folate.

26. The method of claim 24, wherein said moeity bound by a folate receptor comprises methotrexate.

27. The method of claim 19, wherein said internalization moiety comprises a moiety bound by a recycling cell surface receptor.

28. The method of claim 19, wherein said internalization moiety comprises a peptide that enhances non-receptor mediated internalization.

29. The method of claim 20, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activate a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

30. The method of claim 29, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine

(FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

31. The method of claim 20, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radionuclide, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

32. The method of claim 19, wherein the primary targeting agent binds to at least one of the antigens selected from the group consisting of carcinoembryonic antigen (CEA), colon-specific antigen-p (CSAp), CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD45, CD46, CD52, CD66a-d, CD74, CD75, CD80, CD126, B7, HLA-DR, Ia, Ii, HM1.24, MUC 1, MUC 2, MUC 3, MUC 4, NCA, EGFR, HER 2/neu, PAM-4, TAG-72, EGP-1, EGP-2, AFP, HCG, HCG-beta, PLAP, PAP, histone, A3, KS-1, Le(y), S100, PSMA, PSA, tenascin, folate receptor, VEGF, PlGF, ILGF-1 (insulin-like growth factor-1), necrosis antigens, IL-2, IL-6, T101, MAGE, organotropic hormones, oncogene products, cytokeratin, gangliosides and combinations thereof.

33. The method of claim 19, wherein said targetable construct binding moiety of said clearing agent comprises an antibody or antibody fragment.

34. The method of claim 33, wherein said antibody or antibody fragment is an IgG antibody or antibody fragment.

35. The method of claim 34, wherein said IgG antibody comprises an IgG1 antibody.

36. The method of claim 34, wherein said antibody or antibody fragment is an IgG antibody with a C_{H2} deletion that enhances clearance from circulation.

37. The method of claim 34, wherein said antibody or antibody fragment is modified with galactose.

38. The method of claim 21, wherein said clearing agent binds with a plurality of targetable constructs.

39. The method of claim 21, wherein binding of clearing agent to targetable construct stabilizes binding of targetable construct to primary targeting agent at binding sites and enhances clearance of targetable construct not bound to primary targeting agent at binding

sites.

40. The method of claim 21, wherein said targetable construct binding moiety of said clearing agent comprises an antibody or antibody fragment.

41. The method of claim 40, wherein said antibody or antibody fragment is an IgG antibody or antibody fragment.

42. The method of claim 41, wherein said IgG antibody comprises an IgG1 antibody.

43. The method of claim 40, wherein said antibody or antibody fragment is an IgG antibody with a C_{H2} deletion that enhances clearance from circulation.

44. The method of claim 40, wherein said antibody or antibody fragment is modified with galactose.

45. The method of claim 21, wherein said clearing agent binds with a plurality of targetable constructs.

46. The method of claim 21, wherein binding of clearing agent to targetable construct stabilizes binding of targetable construct to primary targeting agent at binding sites and enhances clearance of targetable construct not bound to primary targeting agent at binding sites.

47. The method of claim 19, wherein the mammal has a disease or condition selected from the group consisting of cancer, autoimmune disease, infectious disease, a pathological disease associated with amyloid protein accumulation and cardiovascular lesions.

48. The method of claim 21, wherein said primary targeting agent and said internalization agent are administered simultaneously with said targetable construct, and said clearing agent is administered subsequently to said simultaneous administration or at the same time.

49. A method for increasing contrast in an in vivo visualization system, comprising administering to a mammal a primary targeting agent comprising at least one target binding moiety and at least one targtable construct binding moiety; a targetable construct comprising a primary targeting agent binding moiety, a clearing agent binding moiety, and a visualization moiety; and a clearing agent comprising at least one targetable construct binding moiety, whereby the ratio of circulating targetable construct to bound targetable construct is reduced.

50. The method of claim 49, wherein said clearing agent further comprises an internalization moiety.

51. The method of claim 49, wherein said targetable construct further comprises an internalization agent binding moiety, and said method further comprises administering to said mammal an internalization agent comprising a targetable construct binding moiety and an internalization moiety.

52. The method of claim 49, wherein said targetable construct binding moiety of said clearing agent comprises an antibody or antibody fragment.

53. The method of claim 52, wherein said antibody or antibody fragment is an IgG antibody or antibody fragment.

54. The method of claim 53, wherein said IgG antibody comprises an IgG1 antibody.

55. The method of claim 52, wherein said antibody or antibody fragment is an IgG antibody with a C_{H2} deletion that enhances clearance from circulation.

56. The method of claim 52, wherein said antibody or antibody fragment is modified with galactose.

57. The method of claim 49, wherein said clearing agent binds with a plurality of targetable constructs.

58. The method of claim 49, wherein binding of clearing agent to targetable construct stabilizes binding of targetable construct to primary targeting agent at binding sites and enhances clearance of

targetable construct not bound to primary targeting agent at binding sites.

59. The method of claim 49, wherein said visualization moiety is selected from the group consisting of a photoactive agent or dye, a radionuclide, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

60. The method of claim 49, wherein said primary targeting agent and said clearing agent comprise at least one humanized antibody, **chimeric** antibody, human antibody, murine antibody or an antigen binding fragment thereof. .

61. The method of claim 49, wherein the primary targeting agent binds to at least one of the antigens selected from the group consisting of carcinoembryonic antigen (CEA), colon-specific antigen-p (CSAp), CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD45, CD46, CD52, CD66a-d, CD74, CD75, CD80, CD126, B7, HLA-DR, Ia, Ii, HM1.24, MUC 1, MUC 2, MUC 3, MUC 4, NCA, EGFR, HER 2/neu, PAM-4, TAG-72, EGP-1, EGP-2, AFP, HCG, HCG-beta, PLAP, PAP, histone, A3, KS-1, Le(y), S100, PSMA, PSA, tenascin, folate receptor, VEGF, PlGF, ILGF-1 (insulin-like growth factor-1), necrosis antigens, IL-2, IL-6, T101, MAGE, organotropic hormones, oncogene products, cytokeratin, gangliosides and combinations thereof.

62. The method of claim 49, wherein the mammal has a disease or condition selected from the group consisting of cancer, autoimmune disease, infectious disease, a pathological disease associated with amyloid protein accumulation and cardiovascular lesions.

63. A method for clearing a circulating therapeutic or diagnostic agent, comprising administering a clearing agent to a mammal having said circulating therapeutic or diagnostic agent, wherein said clearing agent specifically binds to at least one moiety on a circulating molecule or complex comprising said therapeutic or diagnostic agent and enhances clearance of said molecule or complex.

64. The method of claim 63, wherein said therapeutic or diagnostic agent is part of a targetable construct comprising at least 2 orthogonal haptens.

65. The method of claims 64, wherein said targetable construct comprises at least 2 copies of an orthogonal hapten.

66. The method of claim 63, wherein said targetable construct comprises a peptide.

67. The method of claim 63, wherein said therapeutic agent is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activate a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

68. The method of claim 67, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil

mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

69. The method of claim 63, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radionuclide, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

70. The method of claim 63, wherein said clearing agent comprises at least one humanized antibody, **chimeric** antibody, human antibody, murine antibody or an antigen binding fragment thereof.

71. The method of claim 63, wherein said clearing agent comprises an antibody or antibody fragment that specifically binds to said molecule or complex.

72. The method of claim 63, wherein said clearing agent binds at least two of said circulating molecules or complexes, thereby crosslinking said molecules or complexes.

73. The method of claim 71, wherein said clearing agent comprises an IgG antibody or antibody fragment.

74. The method of claim 73, wherein said IgG antibody comprises an IgG1 antibody.

75. The method of claim 71, wherein said clearing agent comprises an IgG antibody that has a C_{H2} deletion and is cleared more rapidly from circulation that a corresponding IgG antibody without said deletion.

76. The method of claim 71, wherein said antibody is modified with galactose.

77. The method of claim 71, wherein said clearing agent binds to a plurality of targetable constructs.

78. The method of claim 63, wherein said mammal also has targetable construct bound to primary targeting agent at binding sites and wherein binding of clearing agent to targetable construct stabilizes binding of targetable construct to primary targeting agent at said binding sites.

79. The method of claim 63, wherein the mammal has a disease or condition selected from the group consisting of cancer, autoimmune disease, infectious disease, a pathological disease associated with amyloid protein accumulation and cardiovascular lesions.

80. A tri-specific targetable construct adapted for delivery of a therapeutic or diagnostic moiety, comprising at least one first binding moiety suitable for binding with a separate primary targeting agent; a second binding moiety suitable for binding with a clearing agent; and a third binding moiety suitable for binding with an internalization agent.

81. The construct of claim 80, wherein at least one of said first,

second, and third binding moieties is a hapten.

82. The construct of claim 80, wherein said first, second, and third binding moieties are orthogonal haptens.

83. The construct of claim 80, further comprising a therapeutic or diagnostic moiety.

84. The construct of claim 83, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

85. The method of claim 84, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

86. The construct of claim 83, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

87. The construct of claim 80, wherein said construct bound to a mammalian cell.

88. The construct of claim 80, wherein said construct is in a mammal.

89. A clearing agent suitable for clearing a targetable construct from circulation in a mammal comprising a binding moiety suitable for binding a targetable construct; and an internalization moiety.

90. The agent of claim 89, wherein said internalization moiety comprises

a moiety bound by a folate receptor.

91. The agent of claim 90, wherein said moiety bound by a folate receptor comprises folate.

92. The agent of claim 90, wherein said moiety bound by a folate receptor comprises methotrexate.

93. The agent of claim 89, wherein said internalization moiety comprises a non-receptor mediated internalization peptide.

94. The agent of claim 89, wherein said internalization moiety is bound by a recycling cell surface receptor.

95. The agent of claim 89 further comprising at least 2 binding moieties suitable for binding a targetable construct.

96. The agent of claim 89 further comprising at least one antibody or antibody fragment.

97. The agent of claim 96, wherein said antibody or antibody fragment is an IgG antibody or antibody fragment.

98. The method of claim 97, wherein said IgG antibody comprises an IgG1 antibody.

99. The agent of claim 97, wherein said IgG antibody fragment has a C_{H2} deletion and is cleared more rapidly from circulation that a corresponding antibody not having said deletion.

100. The agent of claim 97, wherein said antibody or antibody fragment is modified with galactose.

101. A molecular complex, comprising a targetable construct comprising at least one primary targeting agent binding moiety and an internalizing agent binding moiety, bound with an internalizing agent.

102. The complex of claim 101, wherein said targetable construct further comprises a clearing agent binding moiety.

103. The complex of claim 101, wherein said internalizing agent comprises a targetable construct binding moiety and an internalization moiety.

104. The complex of claim 103, wherein said internalization moiety comprises a moiety bound by a folate receptor.

105. The complex of claim 104, wherein said moiety bound by a folate receptor comprises folate.

106. The complex of claim 103, wherein said moiety bound by a folate receptor comprises methotrexate.

107. The complex of claim 103, wherein said internalization moiety comprises a non-receptor mediated internalization peptide.

108. The complex of claim 101, wherein said targetable construct further comprises a therapeutic or diagnostic moiety.

109. The construct of claim 108, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

110. The method of claim 109, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase,

leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

111. The construct of claim 108, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

112. The complex of claim 101, wherein said complex is bound to a mammalian cell.

113. The complex of claim 101, wherein said complex is in a mammal.

114. A molecular complex, comprising a targetable construct comprising at least one primary targeting agent binding moiety, a clearing agent binding moiety, and a therapeutic or diagnostic moiety, bound with a clearing agent.

115. The complex of claim 114, wherein said primary targeting agent binding moiety and said clearing agent binding moiety comprise orthogonal haptens.

116. The complex of claim 115 further comprising at least two targetable constructs crosslinked by a clearing agent.

117. The complex of claim 114 further comprising a primary targeting agent, wherein said complex is bound at a target site.

118. The complex of claim 117, wherein said complex is bound to a mammalian cell.

119. The complex of claim 117, wherein said complex is in a mammal.

120. The complex of claim 114, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

121. The method of claim 120, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel,

dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

122. The construct of claim 114, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

123. A molecular complex, comprising a polyvalent primary targeting agent comprising a target binding moiety and a plurality of targetable construct binding moieties, bound with a polyvalent targetable construct comprising a plurality of primary targeting agent binding moieties; a clearing agent binding moiety, and a therapeutic or diagnostic moiety.

124. The complex of claim 123, wherein said plurality of targetable construct binding moieties comprises a plurality of antibody binding domains.

125. The complex of claim 123, wherein said plurality of primary targeting agent binding moieties is two binding moieties.

126. The complex of claim 123, wherein said primary targeting agent binding moieties are orthogonal to said clearing agent binding moiety.

127. The complex of claim 123, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

128. The complex of claim 127, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin

glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

129. The complex of claim 123, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

130. A molecular complex, comprising a primary targeting agent comprising at least one target binding moiety and at least one targetable construct binding moiety; bound with a targetable construct comprising at least one primary targeting agent binding moiety, a clearing agent binding moiety, and a therapeutic or diagnostic binding moiety; and bound with an internalizing agent comprising a targetable construct binding moiety or a clearing agent binding moiety, and an internalizing moiety.

131. The complex of claim 130, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

132. The complex of claim 131, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6 -mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine

streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pb, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

133. The complex of claim 130, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

134. A molecular complex, comprising a primary targeting agent comprising at least one target binding moiety and a therapeutic or diagnostic moiety, bound with an internalization agent comprising a primary targeting agent binding moiety and an internalizing moiety.

135. The complex of claim 134, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide and combinations thereof.

136. The complex of claim 135, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein

said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{212}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

137. The complex of claim 134, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

138. A kit for administration of a therapeutic or diagnostic agent to a mammal, comprising a primary targeting agent comprising at least one target binding moiety and at least one targetable construct binding moiety; a targetable construct comprising at least one primary targeting agent binding moiety, a therapeutic or diagnostic moiety, and a clearing agent binding moiety; and a clearing agent, comprising a targetable construct binding moiety.

139. The kit of claim 138, wherein said therapeutic moiety is selected from the group consisting of a drug, a toxin, a prodrug, a toxin, an enzyme, an enzyme that activates a prodrug to a drug, an enzyme-inhibitor, a nuclease, a hormone, a hormone antagonist, an immunomodulator, an oligonucleotide, a boron compound, a photoactive agent or dye, a radionuclide or combinations thereof.

140. The kit of claim 139, wherein said therapeutic moiety is selected from the group consisting of aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin and analogs thereof, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3',5'-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, nitrogen mustard, ethyleneimine derivative, alkyl sulfonate, nitrosourea, triazene, folic acid analog, anthracycline, COX-2 inhibitor, pyrimidine analog, purine analog, antibiotic, epipodophyllotoxin, platinum coordination complex, vinca alkaloid, substituted urea, methyl hydrazine derivative, adrenocortical suppressant, antagonist, endostatin, cytokine, interleukin, interferon, lymphokine, tumor necrosis factor, antisense oligonucleotide, interference RNA, and combinations thereof, and wherein said radionuclide is selected from the group consisting of ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Se, ^{77}As, ^{86}Y, ^{89}Sr, ^{89}Zr, ^{90}Y, ^{94}Tc, ^{94m}Tc, ^{99}Mo, ^{99m}Tc, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, ^{154} -158Gd, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac

and combinations thereof.

141. The kit of claim 138, wherein said diagnostic moiety is selected from the group consisting of a photoactive agent or dye, a radioopagque material, a contrast agent, a fluorescent compound, an enhancing agent, a light scattering metal colloid particle; a radioimaging metal chelate, a radionuclide and combinations thereof, wherein said radionuclide is selected from the group consisting of ^{18}F, ^{45}Ti, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{99m}Tc ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac and combinations thereof.

142. A kit, comprising a primary targeting agent comprising at least one target binding moiety and at least one internalization agent binding moiety; and an internalization agent comprising an internalization moiety that enhances internalization of a complex comprising said internalization moiety, and a primary targeting agent binding moiety.

143. The kit of claim 142, wherein said internalization agent binds to folate receptor.

144. The kit of claim 142, wherein said internalization moiety comprises folate.

145. The kit of claim 142, wherein said internalization moiety comprises methotrexate.

146. The kit of claim 142, wherein said internalization moiety binds to recycling receptor.

147. A method for synthesizing a DTPA-containing peptide on a solid phase medium, comprising synthesizing said peptide on a solid phase medium; reacting an amino group of said peptide with chloroacetic anhydride, DMAP, DIEA and NMP to provide Intermediate 1, and washing with NMP and IPA; reacting Intermediate 1 with Diethylenetriamine mixed with NMP to provide Intermediate 2, and washing with NMP/IPA; reacting Intermediate 2 with t-Butyl bromoacetate mixed with DIEA and NMP, thereby providing a peptide product with a DTPA moiety.

148. The method of claim 146, wherein the newly synthesized DTPA moiety is protected to allow further elaboration of the peptide.

149. The method of embodiment 148, wherein said further elaboration comprises the removal of Aloc protecting groups from lysine side chains, and coupling trityl-HSG-OH to the lysine side chains, and said method further comprises cleaving the product from said solid phase medium; and purifying said product.

150. The method of claim 147, wherein said peptide is synthesized using the Fmoc procedure, wherein each amino acid is coupled using diisopropylcarbodiimide, followed by a coupling using O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as the activating agents.

151. A method for treating or diagnosing a disease or condition in a subject, comprising administering to said patient a primary targeting agent comprising a target binding moiety and at least one targetable construct binding moiety; a targetable construct comprising at least one primary targeting agent binding moiety, a clearing agent binding moiety, and a therapeutic or diagnostic moiety; and a clearing agent comprising at least one targetable construct binding moiety, wherein said clearing agent enhances retention of said targetable construct at a target site.

L15 ANSWER 6 OF 22 USPATFULL on STN
2004:300206 Immunoconjugates made of egg-yolk antibodies (igy), production and use thereof in diagnoses and therapy.
Bergter, Wolfgang, Hanover, GERMANY, FEDERAL REPUBLIC OF
Kobilke, Hartmut, Damsdorf, GERMANY, FEDERAL REPUBLIC OF
Sroka, Joachim, Damsdorf, GERMANY, FEDERAL REPUBLIC OF
US 2004236076 A1 20041125
APPLICATION: US 2004-478381 A1 20040629 (10)
WO 2002-DE1606 20020503
PRIORITY: DE 2001-123505 20010515
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to immunoconjugates from egg-yolk antibodies (IgY), their production and application in diagnostics and therapy. It is an object of the present invention to propose alternative products to conventional immunoconjugates for diagnostic and therapeutic use. The immunoconjugates in accordance with the invention are superior to monoclonal antibodies and immunosera from mammals, as well as to the IgY compounds from conventional chickens, in respect of purity and antigen targeting. The production of human anti-IgY-antibodies is to be avoided if possible, and the immunoconjugates are to be producible economically in a manner doing justice to animal protection and in large quantities. In accordance with the invention, the object is accomplished by polyclonal IgY conjugates from intact egg-yolk antibodies (IgY), IgY fragments, Fab constructs or humanized egg-yolk antibodies from SPF-chickens, preferably transgenic SPF-chickens. The antigens or antigen fragments are to be conjugated as immunologically effective component with at least one further component which may be a signal agent, an active agent or a booster molecule.

CLM What is claimed is:

1. IgY conjugates for producing a diagnostic or therapeutic agent for non-infectious inflammations, infectious diseases, clotting disturbances, autoimmune diseases and oncological diseases, consisting of a polyclonal IgY compound from eggs of specified pathogen-free (SPF) chickens in accordance with European Pharmakopeia and DAB10 or consisting of a polyclonal IgY compound from eggs of transgenic SPF-chickens, whereby the IgY conjugates contain polyclonal IgY with constant regions of human IgG and additionally consist of at least one signal and/or active agent and/or booster molecule.

2. The IgY conjugates of claim 1, whereby the polyclonal IgY compounds are purified egg-yolk antibodies (IgY), IgY fragments, Fab constructs or **chimeric** egg-yolk antibodies.

3. The IgY conjugates of claim 1, characterized by the fact that the IgY compounds used for the preparation contain humanized egg-yolk antibodies produced by chemical linking of variable regions of aviary antibodies with constant regions of human IgG.

4. The IgY conjugates of claim 1 and 2, characterized by the fact that the IgY compound consists of mono- or bi-valent antibody fragments, preferably Fab or $F(ab)_2$.

5. The IgY conjugates of claim 1 and 2, characterized by the fact that the IgY compound consists of bi-, tri- or polyspecific Fab constructs.

6. The IgY conjugates of claim 1, characterized by the fact that the signal agent is a diagnostic radionuclide, an enzyme, a stain and/or a photosensitizer.

7. The IgY conjugates of claim 1, characterized by the fact that the active agent is a therapeutic radionuclide, a cytostatic agent, a toxin, a chemotherapeutic agent, a prodrug, an enzyme, a photosensitizer and/or a fibrinolytic agent.

8. The IgY conjugates of claim 1, characterized by the fact that the IgY compound is conjugated with a booster molecule, preferably biotin or with a protein of the complement system.

9. The IgY conjugates of one or more of the preceding claims, characterized by the fact that the signal agent or active agent is bonded to the IgY compound by covalent bond or by an auxiliary agent such as a chelating agent, booster molecule and/or molecule of high affinity for the booster molecule, or by the variable region of one or more branches of a bi-, tri- or polyspecific IgY construct.

10. The IgY conjugates of one or more of the preceding claims, characterized by the fact the IgY compound is specific for tumor antigens, for hormones, for RNA- and/or DNA sections, for infectious germs or their (surface)-antigens, for leucocyte-antigens, for intracellular molecules, for receptor molecules or for clotting factors.

11. The IgY conjugates of one or more of the preceding claims, whereby in the sense of pretargeting the immunoconjugates are conjugated in vivo to the signal agent or active agent or booster molecule only after bonding to the specific antigen by way of secondary human anti-IgY antibodies.

12. The IgY conjugates of one or more of the preceding claims, whereby in the sense of pretargeting the immunoconjugates are conjugated in vivo to the signal agent or active agent or booster molecule antigen only

after bonding to the specific antigen by way of secondary human anti-IgY antibodies.

13. The confectioning of IgY conjugates described in claims 1 to 12, whereby they are supplemented by at least a set for quality control by thin-layer-chromatography, separation columns, stands for the apparatus, washing and elution buffers.

14. The confectioning of claim 13, whereby the IgY conjugate is made available in lyophilized form or suspension in connection with additiives such as stabilizers set for an optimum pH value.

15. The confectioning of IgY conjugates of claim 13, whereby and application device is added.

L15 ANSWER 7 OF 22 USPATFULL on STN
2004:239249 Immunoconjugates with an intracellularly-cleavable linkage.
Govindan, Serengulam V., Summit, NJ, UNITED STATES
IMMUNOMEDICS, INC. (U.S. corporation)
US 2004185053 A1 20040923
APPLICATION: US 2003-734589 A1 20031215 (10)
PRIORITY: US 2002-433017P 20021213 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to therapeutic conjugates with improved ability to target various cancer cells containing a targeting moiety and a therapeutic moiety. The targeting and therapeutic moieties are linked via an acid cleavable linkage that increases therapeutic efficacy of the immunoconjugate.

CLM What is claimed is:

1. An immunoconjugate comprising: (a) a targeting moiety; (b) a chemotherapeutic moiety; and (c) a linker binding to the targeting moiety via a thiol group, and to the chemotherapeutic moiety via an intracellularly-cleavable moiety other than a hydrazone.

2. The immunoconjugate according to claim 1, wherein the intracellularly-cleavable moiety is cleavable by intracellular esterases.

3. The immunoconjugate according to claim 2, wherein the intracellularly-cleavable moiety is an ester moiety.

4. The immunoconjugate according to claim 3, wherein said ester moiety is the ester formed from the α-carboxylic acid of an amino acid.

5. The immunoconjugate according to claim 1, wherein the intracellularly-cleavable moiety comprises a peptide bond cleavable by intracellular enzymes.

6. The immunoconjugate according to claim 1, wherein the intracellularly-cleavable moiety comprises an ether bond, susceptible to cleavage under the acidic pH of intracellular compartments.

7. The immunoconjugate according to claim 6, wherein said ether bond is the ether bond formed between the chemotherapeutic agent and said intracellularly-cleavable moiety.

8. The immunoconjugate according to claim 7, wherein said intracellularly-cleavable moiety comprises a tetrahydropyran moiety, a tetrahydrofuran moiety or an orthoester moiety.

9. The immunoconjugate according to claim 1, wherein said linker comprises a thiol-reactive group which links to thiol groups of said targeting moiety.

10. The immunoconjugate according to claim 9, wherein said thiol-reactive group is a maleimide or vinylsulfone which links to thiol groups of said targeting moiety.

11. The immunoconjugate according to claim 1, wherein said linker comprises a thiol group which reacts with a maleimide residue at a lysine side chain of said targeting moiety.

12. The immunoconjugate according to claim 1, wherein said linker further comprises a water-solubilizing moiety between the chemotherapetic moiety and the targeting moiety.

13. The immunoconjugate according to claim 12, wherein said

water-solubilizing moiety is an aminopolycarboxylate.

14. The immunoconjugate according to claim 13, wherein said aminopolycarboxylate residue is selected from the group consisting of DTPA, EDTA, TTHA, benzyl-DTPA, DOTA, benzyl-DOTA, NOTA, benzyl-NOTA, TETA and a N,N'-dialkyl substituted piperazine.

15. The immunoconjugate according to claim 1, wherein said chemotherapeutic moiety is selected from the group consisting of doxorubicin (DOX), epirubicin, morpholinodoxorubicin (morpholino-DOX), cyanomorpholino-doxorubicin (cyanomorpholino-DOX), 2-pyrrolino-doxorubicin (2-PDOX), CPT, CPT-11, SN-38, topotecan, taxanes, geldanamycin, ansamycins, and epothilones.

16. The immunoconjugate according to claim 1, wherein said targeting moiety is an antibody or an antigen binding fragment thereof.

17. The immunoconjugate according to claim 16, wherein said antibody is a monoclonal antibody (mAb).

18. The immunoconjugate according to claim 17, wherein said is a monoclonal antibody that is **multivalent** and/or multispecific.

19. The immunoconjugate according to claim 16, wherein said targeting moiety is a murine, **chimeric**, humanized, or human monoclonal antibody, and said antibody is in intact, fragment (Fab, Fab', $F(ab)_2$, $F(ab')_2$), or sub-fragment (single-chain constructs) form.

20. The immunoconjugate according to claim 18, wherein said targeting moiety is a murine, **chimeric**, humanized, or human monoclonal antibody, and said antibody is in intact, fragment (Fab, Fab', $F(ab)_2$, $F(ab')_2$), or sub-fragment (single-chain constructs) form.

21. The immunoconjugate according to claim 1, wherein said targeting moiety is a monoclonal antibody that is reactive with an antigen or epitope of an antigen expressed on a cancer or malignant cell.

22. The immunoconjugate according to claim 21, wherein said cancer cell is a cell from a hematopoietic tumor, carcinoma, sarcoma, melanoma or a glial tumor.

23. The immunoconjugate according to claim 1, wherein said targeting moiety is a monoclonal antibody that binds to a B-cell lineage antigen, a T-cell antigen, a myeloid lineage antigen and a HLA-DR antigen.

24. The immunoconjugate according to claim 1, wherein said targeting moiety is a monoclonal antibody that binds to an antigen selected from the group consisting of CD74, CD22, epithelial **glycoprotein**-1, MUC1, carcinoembryonic antigen (CEA or CD66e), colon-specific antigen-p, alpha-fetoprotein, CC49, prostate-specific membrane antigen, carbonic anhydrase IX, HER-2/neu, BrE3, CD19, CD20, CD21, CD23, CD33, CD45, CD74, CD80, VEGF, EGF receptor, PlGF, MUC2, MUC3, MUC4, gangliosides, HCG, EGP-2, CD37, HLA-DR, CD30, Ia, A3, A33, Ep-CAM, KS-1, Le(y), S100, PSA, tenascin, folate receptor, Thomas-Friedreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, Ga 733, IL-2, IL-6, T101, MAGE, an antigen that binds to L243, CD66a (BGP), CD66b (CGM6) 66CDc (NCA), 66CDd (CGM1), anti-TAC and combinations thereof.

25. The immunoconjugate according to claim 1, wherein said targeting moiety is selected from the group consisting of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

26. The immunoconjugate according to claim 1, wherein said targeting moiety is a bispecific and/or **bivalent** antibody construct comprising one or more antibodies selected from the group consisting of of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

27. The immunoconjugate according to claim 1, wherein said targeting moiety links to at least one chemotherapeutic moiety.

28. The immunoconjugate according to claim 27, wherein said targeting moiety links to about 7 to 12 said chemotherapeutic moieties.

29. The immunoconjugate according to claim 1, wherein said linker comprises a peptide comprising a thiol-reactive moiety at its N-terminus for linkage to the targeting moiety and one or more side chain amino groups for linkage to at least one chemotherapeutic moiety.

30. The immunoconjugate according to claim 1, wherein said linker comprises a functional group at the N-terminus, a water-solubilizing moiety at the C-terminus, and one or more internal basic amino acids with side chains available for attachment to said chemotherapeutic moiety.

31. The immunoconjugate according to claim 30, wherein said water-solubilizing moiety is selected from the group consisting of DTPA, EDTA, TTHA, benzyl-DTPA, DOTA, benzyl-DOTA, NOTA, benzyl-NOTA and N,N'-dialkyl substituted piperazine.

32. The immunoconjugate of claim 1, wherein said linker is of the formula: ##STR11##

33. The immunoconjugate according to claim 1, wherein said immunoconjugate is in a form suitable for parenteral administration.

34. The immunoconjugate according claim of 29, wherein said chemotherapeutic moiety is selected from the group consisting of doxorubicin (DOX), epirubicin, morpholinodoxorubicin (morpholino-DOX), cyanomorpholino-doxorubicin (cyanomorpholino-DOX), 2-pyrrolino-doxorubicin (2-PDOX), CPT, CPT-11, SN-38, topotecan, taxanes, geldanamycin, ansamycins, and epothilones.

35. The immunoconjugate according to claim 29, wherein said targeting moiety is an antibody or an antigen binding fragment thereof.

36. The immunoconjugate according to claim 35, wherein said antibody is a monoclonal antibody (mAb).

37. The immunoconjugate according to claim 36, wherein said is a monoclonal antibody that is **multivalent** and/or multispecific.

38. The immunoconjugate according to claim 36, wherein said targeting moiety is a murine, **chimeric**, humanized, or human monoclonal antibody, and said antibody is in intact, fragment (Fab, Fab', $F(ab)_2$, $F(ab')_2$), or sub-fragment (single-chain constructs) form.

39. The immunoconjugate according to claim 37, wherein said targeting moiety is a murine, **chimeric**, humanized, or human monoclonal antibody, and said antibody is in intact, fragment (Fab, Fab', $F(ab)_2$, $F(ab')_2$), or sub-fragment (single-chain constructs) form.

40. The immunoconjugate according to claim 29, wherein said targeting moiety is a monoclonal antibody that is reactive with an antigen or epitope of an antigen expressed on a cancer or malignant cell.

41. The immunoconjugate according to claim 40, wherein said cancer cell is a cell from a hematopoietic tumor, carcinoma, sarcoma, melanoma or a glial tumor.

42. The immunoconjugate according to claim 29, wherein said targeting moiety is a monoclonal antibody that binds to a B-cell lineage antigen, a T-cell antigen, a myeloid lineage antigen and a HLA-DR antigen.

43. The immunoconjugate according to claim 29, wherein said targeting moiety is a monoclonal antibody that binds to an antigen selected from the group consisting of CD74, CD22, epithelial **glycoprotein**-1, MUC1, carcinoembryonic antigen (CEA or CD66e), colon-specific antigen-p, alpha-fetoprotein, CC49, prostate-specific membrane antigen, carbonic anhydrase IX, HER-2/neu, BrE3, CD19, CD20, CD21, CD23, CD33, CD45, CD74, CD80, VEGF, EGF receptor, PIGF, MUC2, MUC3, MUC4, gangliosides, HCG, EGP-2, CD37, HLA-DR, CD30, Ia, A3, A33, Ep-CAM, KS-1, Le(y), S100, PSA, tenascin, folate receptor, Thomas-Friedreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, Ga 733, IL-2, IL-6, T101, MAGE, an antigen that binds to L243, CD66a (BGP), CD66b (CGM6) 66CDc (NCA), 66CDd (CGM1), anti-TAC and combinations thereof.

44. The immunoconjugate according to claim 29, wherein said targeting moiety is selected from the group consisting of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

45. The immunoconjugate according to claim 29, wherein said targeting moiety is a bispecific and/or **bivalent** antibody construct comprising one or more antibodies selected from the group consisting of of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

46. The immunoconjugate according to claim 29, wherein said targeting

moiety is selected from the group consisting of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, Immu 31, G250, J591, CC49 and AFP.

47. The immunoconjugate according to claim 29, wherein said targeting moiety is a bispecific and/or **bivalent** antibody construct comprising one or more antibodies selected from the group consisting of of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, Immu 31, G250, J591, and CC49.

48. The immunoconjugate according to claim 29, wherein said targeting moiety links at least one chemotherapeutic moiety.

49. The immunoconjugate according to claim 48, wherein said targeting moiety links to about 7 to 12 said chemotherapeutic moieties.

50. The immunoconjugate according to claim 30, wherein said functional group is a thiol-reactive or an amine-reactive group.

51. A method of treating a malignancy, an autoimmune disease, an infection, or an infectious lesion in a subject comprising administering to said subject a therapeutically effective amount of the immunoconjugate of claim 1.

52. The method according to claim 51, wherein said malignancy is a malignant solid tumor or hematopoietic neoplasm.

53. The method according to claim 51, wherein said immunoconjugate targets an antigen or epitope or iron-siderophore chelate receptor on a pathogen associated with said infection or infectious lesion.

54. The method according to claim 53, wherein said pathogen is selected from the group consisting of a bacterium, fungus, virus, rickettsia, mycoplasma and protozoa.

55. The method according to claim 53, wherein said pathogen is selected from the group consisting of Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhosae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, mycobacterium tuberculosis, rabies virus, influenza virus, cytomegalovirus, herpes simplex virus I, herpes simplex virus II, human serum parvo-like virus, respiratory syncytial virus, varicella-zoster virus, hepatitis B virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, sindbis virus, lymphocytic choriomeningitis virus, wart virus, blue tongue virus, Sendai virus, feline leukemia virus, reo virus, polio virus, simian virus 40, mouse mammary tumor virus, **dengue virus**, rubella virus, Plasmodium falciparum, Plasmodium vivax, Toxoplasma gondii, Trypanosoma rangeli, Trypanosoma cruzi, Trypanosoma rhodesiensei, Trypanosoma brucei, Schistosoma mansoni, Schistosomajapanicum, Babesia bovis, Elmeria tenella, Onchocerca volvulus, Leishmania tropica, Trichinella spiralis, Theileria parva, Taenia hydatigena, Taenia ovis, Taenia saginata, Echinococcus granulosus, Mesocestoides corti, Mycoplasma arthritidis, M hyorhinis, M. orale, M. arginini, Acholeplasma laidlawii, M. salivarium and M. pneumoniae.

56. The method according to claim 51, wherein said autoimmune disease is a class III autoimmune disease.

57. The method according to claim 56, wherein said class III autoimmune disease is selected from the group consisting of immune-mediated thrombocytopenias, dermatomyositis, Sjogren's syndrome, multiple sclerosis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, rheumatoid arthritis, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis ubiterans, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis and fibrosing alveolitis.

58. The method of claim 51, wherein said immunoconjugate is administered parenterally.

59. The method of claim 51, wherein said targeting moiety is a monoclonal antibody that binds to a B-cell lineage antigen, a T-cell antigen, a myeloid lineage antigen and a HLA-DR antigen.

60. The method according to claim 51, wherein said targeting moiety is a monoclonal antibody that binds to an antigen selected from the group consisting of CD74, CD22, epithelial **glycoprotein**-1, MUC1, carcinoembryonic antigen (CEA or CD66e), colon-specific antigen-p, alpha-fetoprotein, CC49, prostate-specific membrane antigen, carbonic anhydrase IX, HER-2/neu, BrE3, CD19, CD20, CD21, CD23, CD33, CD45, CD74, CD80, VEGF, EGF receptor, PlGF, MUC2, MUC3, MUC4, gangliosides, HCG, EGP-2, CD37, HLA-DR, CD30, Ia, A3, A33, Ep-CAM, KS-1, Le(y), S100, PSA, tenascin, folate receptor, Thomas-Friedreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, Ga 733, IL-2, IL-6, T101, MAGE, an antigen that binds to L243, CD66a (BGP), CD66b (CGM6) 66CDc (NCA), 66CDd (CGM1), anti-TAC and combinations thereof.

61. The method according to claim 51, wherein said targeting moiety is selected from the group consisting of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

62. The method according to claim 51, wherein said targeting moiety is a bispecific and/or **bivalent** antibody construct comprising one or more antibodies selected from the group consisting of of LL1, LL2, hA20, 1F5, L243, RS7, PAM-4, MN-14, Mu-9, AFP-31, G250, J591, CC49 and Immu 31.

L15 ANSWER 8 OF 22 USPATFULL on STN
2004:184069 Death domain containing receptor 5.
Ni, Jian, Rockville, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Human Genome Sciences, Inc., Rockville, MD (U.S. corporation)
US 2004141952 A1 20040722
APPLICATION: US 2004-774622 A1 20040210 (10)
PRIORITY: US 1999-148939P 19990813 (60)
US 1999-133238P 19990507 (60)
US 1999-132498P 19990504 (60)
US 1997-54021P 19970729 (60)
US 1997-40846P 19970317 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel Death Domain Containing Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid molecules are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying antagonists and antagonists of DR5 activity.
CLM What is claimed is:
1. A method for treating graft versus host disease, viral infection, cancer, leukemia, immunodeficiency, or an autoimmune disorder comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO:2; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

2. The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 1 to 133 of SEQ ID NO:2.

3. The method of claim 1, wherein said antibody is a monoclonal antibody.

4. The method of claim 1, wherein said antibody is a polyclonal antibody.

5. The method of claim 1, wherein said antibody is a **chimeric** antibody.

6. The method of claim 1, wherein said antibody is a humanized antibody.

7. The method of claim 1, wherein said antibody is a single-chain Fv

antibody.

8. The method of claim 1, wherein said antibody is an Fab antibody fragment.

9. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at the same time.

10. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at different times.

11. The method of claim 1, wherein said second therapeutic agent is TRAIL.

12. The method of claim 1, wherein said tumor necrosis factor blocking agent comprises an antibody which binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

13. The method of claim 1, wherein said immunosuppressive agent is selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

14. The method of claim 1, wherein said cytokine is selected from the group consisting of (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

15. A composition comprising: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO:2; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

16. The composition of claim 15, which further comprises a pharmaceutically acceptable carrier or excipient.

17. An isolated polypeptide comprising an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO:2; wherein said polypeptide is covalently attached to polyethylene glycol, said polyethylene glycol having an average molecule weight selected from the group consisting of 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, and 20,000.

18. The polypeptide of claim 17, comprising an amino acid sequence at least 95% identical to amino acids 1 to 133 of SEQ ID NO:2.

19. The polypeptide of claim 18, wherein said amino acid sequence comprises amino acids 1 to 133 of SEQ ID NO:2.

20. The polypeptide of claim 17, wherein said polypeptide has an average degree of substitution with polyethylene glycol which falls within a range selected from the group consisting of 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, and 10-12.

21. The polypeptide of claim 17, which is produced by a recombinant host cell.

22. The polypeptide of claim 21, wherein said recombinant host cell which is a eukaryotic host cell.

23. The polypeptide of claim 17, which comprises a heterologous polypeptide.

24. The polypeptide of claim 23, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

25. A composition comprising the polypeptide of claim 17 and a pharmaceutically acceptable carrier.

26. An isolated polynucleotide comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO:2.

27. The polynucleotide of claim 26, comprising a nucleic acid encoding

an amino acid sequence at least 95% identical to amino acids 1 to 133 of SEQ ID NO:2.

28. The polynucleotide of claim 27, comprising a nucleic acid encoding amino acids 1 to 133 of SEQ ID NO:2.

29. The polynucleotide of claim 26, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids 1 to 360 of SEQ ID NO:2.

30. The polynucleotide of claim 29, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to amino acids 1 to 360 of SEQ ID NO:2.

31. The polynucleotide of claim 30, comprising a nucleic acid encoding amino acids 24 to 468 of SEQ ID NO:2.

32. The polynucleotide of claim 29, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids -51 to 360 of SEQ ID NO:2.

33. The polynucleotide of claim 32, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to amino acids -51 to 360 of SEQ ID NO:2.

34. The polynucleotide of claim 33, comprising a nucleic acid encoding amino acids -51 to 360 of SEQ ID NO:2.

35. The polynucleotide of claim 26, further comprising a heterologous polynucleotide.

36. The polynucleotide of claim 35, wherein said heterologous polynucleotide encodes a heterologous polypeptide.

37. The polynucleotide of claim 26, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

38. A method of producing a vector which comprises inserting the polynucleotide of claim 26 into a vector.

39. A vector comprising the polynucleotide of claim 26.

40. The vector of claim 39, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

41. A host cell comprising the polynucleotide of claim 26.

42. The host cell of claim 41, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

43. A method of producing a polypeptide which comprises culturing the host cell of claim 32 under conditions such that said polypeptide is expressed, and recovering said polypeptide.

44. An isolated polynucleotide comprising a nucleic acid encoding an amino acid sequence at least 90% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

45. The polynucleotide of claim 44, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

46. The polynucleotide of claim 45, comprising a nucleic acid encoding the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

47. The polynucleotide of claim 44, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

48. The polynucleotide of claim 47, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

49. The polynucleotide of claim 48, comprising a nucleic acid a nucleic

acid encoding the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

50. The polynucleotide of claim 44, further comprising a heterologous polynucleotide.

51. The polynucleotide of claim 50, wherein said heterologous polynucleotide encodes a heterologous polypeptide.

52. The polynucleotide of claim 51, wherein said heterologous polynucleotide encodes an Fc portion of an antibody.

53. A method of producing a vector which comprises inserting the polynucleotide of claim 44 into a vector.

54. A vector comprising the polynucleotide of claim 44.

55. The vector of claim 54, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

56. A host cell comprising the polynucleotide of claim 44.

57. The host cell of claim 56, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

58. A method of producing a polypeptide which comprises culturing the host cell of claim 57 under conditions such that said polypeptide is expressed, and recovering said polypeptide.

59. An isolated polypeptide comprising an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO:2.

60. The polypeptide of claim 59, wherein said amino acid sequence is at least 95% identical to amino acids 1 to 133 of SEQ ID NO:2.

61. The polypeptide of claim 60, wherein said amino acid sequence comprises amino acids 1 to 133 of SEQ ID NO:2.

62. The polypeptide of claim 59, wherein said amino acid sequence is at least 90% identical to amino acids 1 to 133 of SEQ ID NO:2.

63. The polypeptide of claim 62, wherein said amino acid sequence is at least 95% identical to amino acids 1 to 360 of SEQ ID NO:2.

64. The polypeptide of claim 63, wherein said amino acid sequence comprises amino acids 1 to 360 of SEQ ID NO:2.

65. The polypeptide of claim 62, wherein said amino acid sequence is at least 90% identical to amino acids -51 to 360 of SEQ ID NO:2.

66. The polypeptide of claim 65, wherein said amino acid sequence is at least 95% identical to amino acids -51 to 360 of SEQ ID NO:2.

67. The polypeptide of claim 66, wherein said amino acid sequence comprises amino acids -51 to 360 of SEQ ID NO:2.

68. The polypeptide of claim 59, which is produced by a recombinant host cell.

69. The polypeptide of claim 68, wherein said recombinant host cell which is a eukaryotic host cell.

70. The polypeptide of claim 59, which comprises a heterologous polypeptide.

71. The polypeptide of claim 70, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

72. A composition comprising the polypeptide of claim 59 and a pharmaceutically acceptable carrier.

73. An isolated polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

74. The polypeptide of claim 73, which comprises an amino acid sequence at least 95% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

75. The polypeptide of claim 74, which comprises the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

76. The polypeptide of claim 73, which comprises an amino acid sequence at least 90% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

77. The polypeptide of claim 76, which comprises an amino acid sequence at least 95% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

78. The polypeptide of claim 77, which comprises the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

79. The polypeptide of claim 73, which is produced by a recombinant host cell.

80. The polypeptide of claim 79, wherein said recombinant host cell which is a eukaryotic host cell.

81. The polypeptide of claim 73, which comprises a heterologous polypeptide.

82. The polypeptide of claim 81, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

83. A composition comprising the polypeptide of claim 73 and a pharmaceutically acceptable carrier.

84. An isolated antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO:2.

85. The antibody of claim 84, wherein said antibody is a monoclonal antibody.

86. The antibody of claim 84, wherein said antibody is a polyclonal antibody.

87. The antibody of claim 84, wherein said antibody is an Fab antibody fragment.

88. The antibody of claim 84, wherein said antibody is an F(ab')2 antibody fragment.

89. A method for treating a disease or condition selected from the group consisting of: (a) cancer, (b) inflammation; (c) an autoimmune disease; and (d) graft v. host disease, wherein said method comprises administering to an individual a therapeutically effective amount of the antibody of claim 84.

90. A composition comprising the antibody of claim 84 and a pharmaceutically acceptable carrier.

91. An isolated antibody which binds to a polypeptide consisting of the amino acid sequence of the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920.

92. The antibody of claim 91, wherein said antibody is a monoclonal antibody.

93. The antibody of claim 91, wherein said antibody is a polyclonal antibody.

94. The antibody of claim 91, wherein said antibody is an Fab antibody fragment.

95. The antibody of claim 91, wherein said antibody is an F(ab')2 antibody fragment.

96. A method for treating a disease or condition selected from the group consisting of: (a) cancer, (b) inflammation; (c) an autoimmune disease; and (d) graft versus host disease, wherein said method comprises administering to an individual a therapeutically effective amount of the antibody of claim 91.

97. A composition comprising the antibody of claim 91 and a pharmaceutically acceptable carrier.

L15 ANSWER 9 OF 22 USPATFULL on STN
2004:177787 Death domain containing receptor 5.
Ni, Jian, Germantown, MD, UNITED STATES
Gentz, Reiner L., Belo Horizonte, BRAZIL
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Human Genome Sciences, Inc. (U.S. corporation)
US 2004136951 A1 20040715
APPLICATION: US 2003-648825 A1 20030827 (10)
PRIORITY: US 2002-413747P 20020927 (60)
US 2002-406307P 20020828 (60)
US 1999-148939P 19990813 (60)
US 1999-133238P 19990507 (60)
US 1999-132498P 19990504 (60)
US 1997-54021P 19970729 (60)
US 1997-40846P 19970317 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel Death Domain Containing Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid molecules are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying antagonists and antagonists of DR5 activity. The invention also relates to the treatment of diseases associated with reduced or increased levels of apoptosis using antibodies specific for DR5, which maybe agonists and/or antagonists of DR5 activity.

CLM What is claimed is:

1. A method for treating graft versus host disease, viral infection, immunodeficiency, or an autoimmune disorder comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 411 of SEQ ID NO:2; (ii) amino acids 52 to 411 of SEQ ID NO:2; (iii) amino acids 52 to 184 of SEQ ID NO:2; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine. A The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 52 to 184 of SEQ ID NO:2.

3. The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

4. The method of claim 1, wherein said antibody is an agonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

5. The method of claim 1, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

6. The method of claim 1, wherein said antibody is an antagonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

7. The method of claim 1, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

8. The method of claim 1, wherein said antibody is an agonistic antibody.

9. The method of claim 1, wherein said antibody is a monoclonal antibody.

10. The method of claim 1, wherein said antibody is a polyclonal antibody.

11. The method of claim 1, wherein said antibody is a **chimeric** antibody.

12. The method of claim 1, wherein said antibody is a human antibody.

13. The method of claim 1, wherein said antibody is a humanized antibody.

14. The method of claim 1, wherein said antibody is a single-chain Fv antibody.

15. The method of claim 1, wherein said antibody is an Fab antibody fragment.

16. The method of claim 1, wherein said antibody is pegylated.

17. The method of claim 1, wherein said antibody is fused to a heterologous polypeptide.

18. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at the same time.

19. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at different times.

20. The method of claim 1, wherein said second therapeutic agent is TRAIL.

21. The method of claim 1, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

22. The method of claim 1, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

23. The method of claim 1, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

24. The method of claim 1, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

25. The method of claim 1, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade.TM.); and (d) a smac peptide or polypeptide.

26. A method for treating cancer comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 411 of SEQ ID NO:2; (ii) amino acids 52 to 411 of SEQ ID NO:2; (iii) amino acids 52 to 184 of SEQ ID NO:2; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (viii) a chemotherapeutic agent; and (viii) a cytokine.

27. The method of claim 26, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 52 to 184 of SEQ ID NO:2.

28. The method of claim 26, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

29. The method of claim 26, wherein said antibody is an agonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

30. The method of claim 26, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

31. The method of claim 26, wherein said antibody is an antagonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

32. The method of claim 26, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

33. The method of claim 26, wherein said antibody is an agonistic antibody.

34. The method of claim 26, wherein said antibody is a monoclonal antibody.

35. The method of claim 26, wherein said antibody is a polyclonal antibody.

36. The method of claim 26, wherein said antibody is a **chimeric** antibody.

37. The method of claim 26, wherein said antibody is a human antibody.

38. The method of claim 26, wherein said antibody is a humanized antibody.

39. The method of claim 26, wherein said antibody is a single-chain Fv antibody.

40. The method of claim 26, wherein said antibody is an Fab antibody fragment.

41. The method of claim 26, wherein said antibody is pegylated.

42. The method of claim 26, wherein said antibody is fused to a heterologous polypeptide.

43. The method of claim 26, wherein said first and second therapeutic agents are administered to the individual at the same time.

44. The method of claim 26, wherein said first and second therapeutic agents are administered to the individual at different times.

45. The method of claim 26, wherein said second therapeutic agent is TRAIL.

46. The method of claim 26, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

47. The method of claim 26, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

48. The method of claim 26, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) L-15; and (k) IFN-γ.

49. The method of claim 26, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

50. The method of claim 26, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade.TM.); and (d) a smac peptide or polypeptide.

51. A composition comprising: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 411 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (ii) amino acids 52 to 411 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (iii) amino acids 52 to 184 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No.97920, wherein said polypeptide is expressed on the surface of a cell; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No.97920, wherein said polypeptide is expressed on the surface of a cell; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920, wherein said polypeptide is expressed on the surface of a cell; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

52. The composition of claim 51, which further comprises a pharmaceutically acceptable carrier.

53. The composition of claim 51, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 52 to 184 of SEQ ID NO:2.

54. The composition of claim 51, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

55. The composition of claim 51, wherein said antibody is an agonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

56. The composition of claim 51, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

57. The composition of claim 51, wherein said antibody is an antagonist of a polypeptide comprising amino acids 52 to 184 of SEQ ID NO:2.

58. The composition of claim 51, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920.

59. The composition of claim 51, wherein said antibody is an agonistic antibody.

60. The composition of claim 51, wherein said antibody is a monoclonal antibody.

61. The composition of claim 51, wherein said antibody is a polyclonal antibody.

62. The composition of claim 51, wherein said antibody is a **chimeric** antibody.

63. The composition of claim 51, wherein said antibody is a human antibody.

64. The composition of claim 51, wherein said antibody is a humanized antibody.

65. The composition of claim 51, wherein said antibody is a single-chain Fv antibody.

66. The composition of claim 51, wherein said antibody is an Fab antibody fragment.

67. The composition of claim 51, wherein said antibody is pegylated.

68. The composition of claim 51, wherein said antibody is fused to a heterologous polypeptide.

69. The composition of claim 51, wherein said second therapeutic agent is TRAIL.

70. The composition of claim 51, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

71. The composition of claim 51, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

72. The composition of claim 51, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

73. The composition of claim 51, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

74. The composition of claim 51, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade.TM.); and (d) a smac peptide or polypeptide.

75. A method for treating a disease or condition selected from the group consisting of: (a) cancer; (b) inflammation; (c) an autoimmune disease; and (d) graft v. host disease, wherein said method comprises administering to an individual in need thereof, a therapeutically effective amount of the composition of claim 51.

76. A method for causing death of a cell, which expresses on its surface a polypeptide having an amino acid sequence selected from the group consisting of: (a) amino acids 52 to 411 of SEQ ID NO:2; and (b) amino acids 52 to 184 of SEQ ID NO:2; wherein said method comprises contacting said cell with the composition of claim 51.

77. A method for causing death of a cell, which expresses on its surface a polypeptide having an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; (b) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; and (c) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920; wherein said method comprises contacting said cell with the composition of claim 51.

L15 ANSWER 10 OF 22 USPATFULL on STN
2004:177786 Death domain containing receptor 4.
Ni, Jian, Germantown, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Gentz, Reiner L., Belo-Horizonte, BRAZIL
Human Genome Sciences, Inc. (U.S. corporation)The Regents of the University of Michigan (U.S. corporation)
US 2004136950 A1 20040715
APPLICATION: US 2003-648786 A1 20030827 (10)
PRIORITY: US 2002-413861P 20020927 (60)
US 2002-406922P 20020830 (60)
US 1999-132922P 19990506 (60)
US 1997-37829P 19970205 (60)
US 1997-35722P 19970128 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel Death Domain Containing Receptor-4 (DR4) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR4 proteins. DR4 polypeptides are also

provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR4 activity and methods for using DR4 polynucleotides and polypeptides. The invention also relates to the treatment of diseases associated with reduced or increased levels of apoptosis using antibodies specific for DR4, which may be agonists and/or antagonists of DR4 activity.

CLM What is claimed is:

1. A method for treating graft versus host disease, viral infection, immunodeficiency, or an autoimmune disorder comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 468 of SEQ ID NO:2; (ii) amino acids 24 to 468 of SEQ ID NO:2; (iii) amino acids 24 to 238 of SEQ ID NO:2; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

2. The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 24 to 238 of SEQ ID NO:2.

3. The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

4. The method of claim 1, wherein said antibody is an agonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

5. The method of claim 1, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

6. The method of claim 1, wherein said antibody is an antagonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

7. The method of claim 1, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

8. The method of claim 1, wherein said antibody is an agonistic antibody.

9. The method of claim 1, wherein said antibody is a monoclonal antibody.

10. The method of claim 1, wherein said antibody is a polyclonal antibody.

11. The method of claim 1, wherein said antibody is a **chimeric** antibody.

12. The method of claim 1, wherein said antibody is a human antibody.

13. The method of claim 1, wherein said antibody is a humanized antibody.

14. The method of claim 1, wherein said antibody is a single-chain Fv antibody.

15. The method of claim 1, wherein said antibody is an Fab antibody fragment.

16. The method of claim 1, wherein said antibody is pegylated.

17. The method of claim 1, wherein said antibody is fused to a heterologous polypeptide.

18. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at the same time.

19. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at different times.

20. The method of claim 1, wherein said second therapeutic agent is TRAIL.

21. The method of claim 1, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

22. The method of claim 1, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

23. The method of claim 1, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

24. The method of claim 1, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

25. The method of claim 1, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) Ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade.TM.); and (d) a smac peptide or polypeptide.

26. A method for treating cancer comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 468 of SEQ ID NO:2; (ii) amino acids 24 to 468 of SEQ ID NO:2; (iii) amino acids 24 to 238 of SEQ ID NO:2; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (viii) a chemotherapeutic agent; and (viii) a cytokine.

27. The method of claim 26, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 24 to 238 of SEQ ID NO:2.

28. The method of claim 26, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

29. The method of claim 26, wherein said antibody is an agonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

30. The method of claim 26, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

31. The method of claim 26, wherein said antibody is an antagonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

32. The method of claim 26, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

33. The method of claim 26, wherein said antibody is an agonistic antibody.

34. The method of claim 26, wherein said antibody is a monoclonal

antibody.

35. The method of claim 26, wherein said antibody is a polyclonal antibody.

36. The method of claim 26, wherein said antibody is a **chimeric** antibody.

37. The method of claim 26, wherein said antibody is a human antibody.

38. The method of claim 26, wherein said antibody is a humanized antibody.

39. The method of claim 26, wherein said antibody is a single-chain Fv antibody.

40. The method of claim 26, wherein said antibody is an Fab antibody fragment.

41. The method of claim 26, wherein said antibody is pegylated.

42. The method of claim 26, wherein said antibody is fused to a heterologous polypeptide.

43. The method of claim 26, wherein said first and second therapeutic agents are administered to the individual at the same time.

44. The method of claim 26, wherein said first and second therapeutic agents are administered to the individual at different times.

45. The method of claim 26, wherein said second therapeutic agent is TRAIL.

46. The method of claim 26, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

47. The method of claim 26, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

48. The method of claim 26, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-S; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

49. The method of claim 26, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

50. The method of claim 26, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) Ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade.TM.); and (d) a smac peptide or polypeptide.

51. A composition comprising: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide selected from the group consisting of: (i) amino acids 1 to 468 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (ii) amino acids 24 to 468 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (iii) amino acids 24 to 238 of SEQ ID NO:2, wherein said polypeptide is expressed on the surface of a cell; (iv) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853, wherein said polypeptide is expressed on the surface of a cell; (v) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853, wherein said polypeptide is expressed on the surface of a cell; and (vi) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853, wherein said polypeptide is expressed on the surface of a cell; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor

blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

52. The composition of claim 51, which further comprises a pharmaceutically acceptable carrier.

53. The composition of claim 51, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids 24 to 238 of SEQ ID NO:2.

54. The composition of claim 51, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

55. The composition of claim 51, wherein said antibody is an agonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

56. The composition of claim 51, wherein said antibody is an agonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

57. The composition of claim 51, wherein said antibody is an antagonist of a polypeptide comprising amino acids 24 to 238 of SEQ ID NO:2.

58. The composition of claim 51, wherein said antibody is an antagonist of a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853.

59. The composition of claim 51, wherein said antibody is an agonistic antibody.

60. The composition of claim 51, wherein said antibody is a monoclonal antibody.

61. The composition of claim 51, wherein said antibody is a polyclonal antibody.

62. The composition of claim 51, wherein said antibody is a **chimeric** antibody.

63. The composition of claim 51, wherein said antibody is a human antibody.

64. The composition of claim 51, wherein said antibody is a humanized antibody.

65. The composition of claim 51, wherein said antibody is a single-chain Fv antibody.

66. The composition of claim 51, wherein said antibody is an Fab antibody fragment.

67. The composition of claim 51, wherein said antibody is pegylated.

68. The composition of claim 51, wherein said antibody is fused to a heterologous polypeptide.

69. The composition of claim 51, wherein said second therapeutic agent is TRAIL.

70. The composition of claim 51, wherein said second therapeutic agent is a tumor necrosis factor blocking agent comprising an antibody that binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

71. The composition of claim 51, wherein said second therapeutic agent is an immunosuppressive agent selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

72. The composition of claim 51, wherein said second therapeutic agent is a cytokine selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

73. The composition of claim 51, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) an alkylating agent; (b) an antimetabolite; (c) a farnesyl transferase inhibitor; (d) a mitotic spindle inhibitor; (e) a nucleotide analog; (f) a platinum analog; and (g) a topoisomerase inhibitor.

74. The composition of claim 51, wherein said second therapeutic agent is a chemotherapeutic agent selected from the group consisting of: (a) Ibritumomab tiuxetan (Zevalin.TM.); (b) imatinib mesylate (Gleevec®); (c) bortezomib (Velcade®); and (d) a smac peptide or polypeptide.

75. A method for treating a disease or condition selected from the group consisting of: (a) cancer; (b) inflammation; (c) an autoimmune disease; and (d) graft v. host disease, wherein said method comprises administering to an individual in need thereof, a therapeutically effective amount of the composition of claim 51.

76. A method for causing death of a cell, which expresses on its surface a polypeptide having an amino acid sequence selected from the group consisting of: (a) amino acids 24 to 468 of SEQ ID NO:2; and (b) amino acids 24 to 238 of SEQ ID NO:2; wherein said method comprises contacting said cell with the composition of claim 51.

77. A method for causing death of a cell, which expresses on its surface a polypeptide having an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; (b) the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; and (c) the amino acid sequence of the extracellular domain of the polypeptide encoded by the cDNA contained in ATCC Deposit No. 97853; wherein said method comprises contacting said cell with the composition of claim 51.

L15 ANSWER 11 OF 22 USPATFULL on STN
2004:57028 Polymeric delivery systems.
Griffiths, Gary L., Morristown, NJ, UNITED STATES
Goldenberg, David M., Medham, NJ, UNITED STATES
Hansen, Hans J., Picayune, MS, UNITED STATES
Immunomedics, Inc. (U.S. corporation)
US 2004043030 A1 20040304
APPLICATION: US 2003-456580 A1 20030609 (10)
PRIORITY: US 2001-308605P 20010731 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method of targeting an agent towards a targeting site in a tissue comprising administering a multi-specific antibody or antibody fragment comprising a targeting arm and a capture arm that binds to a polymer conjugate, and administering a polymer conjugate to the tissue. The present invention also relates to a kit for targeting a target site within a comprising a multi-specific antibody or antibody fragment comprising a targeting arm and a capture arm that binds to a polymer conjugate, and a polymer conjugate.
CLM What is claimed is:
1. A method for diagnosing or treating a disease or disorder comprising: (a) administering to a tissue a multi-specific antibody or antibody fragment, comprising a targeting arm that binds to an antigen on said target site, and a capture arm that binds to a polymer conjugate; and (b) administering to said tissue a polymer conjugate that binds to said capture arm, said polymer conjugate comprising a polymer conjugated to a diagnostic or therapeutic agent.

2. The method of claim 1, wherein said disease or disorder is selected from the group consisting of a cancer, cardiovascular lesion, an inflammatory disease, neurodegenerative disease, metabolic disease, and an infectious disease.

3. The method of claim 2, wherein said cancer is selected from the group consisting of a solid tumor, a B-cell malignancy and a T-cell malignancy.

4. The method of claim 3, wherein said disease or disorder is a B-cell malignancy selected from the group consisting of indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, and multiple myeloma.

5. The method of claim 3, wherein said solid tumor is selected from the group consisting of a melanoma, carcinoma, glioma and sarcoma.

6. The method of claim 5, wherein said carcinoma is selected from the group consisting of renal carcinoma, lung carcinoma, intestinal carcinoma, and stomach carcinoma.

7. The method of claim 2, wherein said cancer is selected from the group consisting of esophageal, gastric, colonic, rectal, pancreatic, lung, breast, ovarian, urinary bladder, endometrial, cervical, testicular, renal, adrenal and liver cancer.

8. The method of claim 2, wherein said cardiovascular lesion is selected from the group consisting of an infarct, clot, embolus, atherosclerotic plaque, and ischemia.

9. The method of claim 2, wherein said neurodegenerative disease is Alzheimer's disease.

10. The method of claim 2, wherein said metabolic disease is amyloidosis and said antibody binds amyloid.

11. The method of claim 1, wherein said disease or disorder is displaced or ectopic normal tissue.

12. The method of claim 11, wherein said tissue is selected from the group consisting of endometrium, thymus, spleen and parathyroid.

13. The method of claim 1, wherein said method can be used for normal tissue ablation.

14. The method of claim 11, wherein said tissue is selected from the group consisting of bone marrow and spleen.

15. The method of claim 1, wherein said disease or disorder is an autoimmune disease.

16. The method of claim 15, wherein said autoimmune disease is selected from the group consisting of myasthenia gravis, lupus nephritis, lupus erythematosus, and rheumatoid arthritis, Class III autoimmune diseases such as immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpura and chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sjogren's syndrome, multiple sclerosis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis ubiterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis and fibrosing alveolitis.

17. The method of claim 2, wherein said infectious disease is selected from the group consisting of a bacterial, fungal, parasitic and viral lesion.

18. The method of claim 17, wherein said infectious disease is caused by a fungus selected from the group consisting of Microsporum, Trichophyton, Epidermophyton, Ssporothrix schenckii, Cyrptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis, and Candida albicans.

19. The method of claim 17, wherein said infectious disease is caused by a virus selected from the group consisting of human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, **Dengue virus**, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus and blue tongue virus.

20. The method of claim 17, wherein said infectious disease is caused by a bacterium selected from the group consisting of Anthrax bacillus, Streptococcus agalactiae, Legionella pneumophilia, Streptococcus

pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis and Tetanus toxin.

21. The method of claim 17, wherein said infectious disease is caused by a parasite selected from the group consisting of a helminth or a malarial parasite.

22. The method of claim 2, wherein said infectious disease is caused by a protozoa selected from the group consisting of Plasmodium falciparum, Plasmodium vivax, Toxoplasma gondii, Trypanosoma rangeli, Trypanosoma cruzi, Trypanosoma rhodesiensei, Trypanosoma brucei, Schistosoma mansoni, Schistosoma japanicum, Babesia bovis, Elmeria tenella, Onchocerca volvulus, Leishmania tropica, Trichinella spiralis, Onchocerca volvulus, Theileria parva, Taenia hydatigena, Taenia ovis, Taenia saginata, Echinococcus granulosus and Mesocestoides corti.

23. The method of claim 2, wherein said infectious disease is caused by a mycoplasma selected from the group consisting of Mycoplasma arthritidis, Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma arginini, Acholeplasma laidlawii, Mycoplasma salivarum, and Mycoplasma pneumoniae.

24. The method of claim 1, wherein said antigen is selected from the group consisting of carcinoembryonic antigen (CEA), HER-2/neu, epidermal growth factor receptor (EGFR), VEGF, placental growth factor (PLGF), tenascin, EGP-1, EGP-2, CD19, CD20, CD22, CD21, CD23, CD30, CD33, CD45, CD80, and CD74. α-fetoprotein, A3, CA125, colon-specific antigen-p (CSAp), folate receptor, HLA-DR, human chorionic gonadrotropin, Ia, IL-2, insulin-like growth factor, KS-1, Le(y), MAGE, MUC1, MUC2, MUC3, MUC4; NCA66, necrosis antigens, PAM-4, prostatic acid phosphatase (PAP), Pr1, prostate specific antigen (PSA), PSMA, S100, T101, TAC, IL-6 and TAG-72.

25. The method of claim 2, wherein said cancer is selected from the group consisting of a CEA-expressing tumor or a CD20-expressing malignancy.

26. The method of claim 25, wherein said CD20-expressing malignancy is a B-cell lymphoma or leukemia.

27. The method of claim 1, wherein said polymer conjugate has a general formula comprising (polymer backbone)-$(agent)_m$, where m is an integer.

28. The method of claim 1, wherein said polymer conjugate further comprises a recognition hapten conjugated to said polymer.

29. The method of claim 1, wherein said polymer conjugate has a general formula comprising $(recognition\ hapten)_n$-(polymer backbone)-$(agent)_m$, where n and m are integers.

30. The method of claim 29, wherein said recognition hapten is selected from the group consisting of: diethylenetriaminepentaacetic acid (DTPA), a metal complex of DTPA, 1,4,7,10-tetrazacyclododecane-N,N', N'", N'"-tetraacetic acid (DOTA), a metal complex of DOTA, N,N'-di[2-hydroxy-5-(ethylene-.E-backward.-carboxy)benzyl]ethylenediamin e N,N'-diacetic acid (HBED), a metal complex of HBED, fluorescein, 2,4-dinitrophenyl-derivatives, biotin and histaminyl-succinyl-glycine.

31. The method of claim 1, wherein said multi-specific antibody or antibody fragment is radiolabeled.

32. The method of claim 31, wherein said multi-specific antibody or antibody fragment is radiolabeled with a radionuclide selected from the group consisting of F-18, P-32, Sc-47, Cu-62, Cu-64, Cu-67, Ga-67, Ga-68, Y-86, Y-90, Zr-89, Tc-99m, Pd-109, Ag-111, In-111, I-123, I-125, I-131, Sm-153, Gd-155, Gd-157, Tb-161, Lu-177, Re-186, Re-188, Pt-197, Pb-212, Bi-212, Bi-213, Ra-223, Ac-225, As-72, As-77, At-211, Au-198, Au-199, Bi-212, Br-75, Br-76B, C-11, Co-55Co, Dy-166, Er-169, F-18, Fe-52, Fe-59, Ga-67, Ga-68, Gd -154-158, Ho-166, I-120, I-121, I-124, In-110, In-111, Iril94, Lu-177, Mn-51, Mn-52, Mo-99, N-13, O-15, P-32, P-33, Pb-211, Pb-212, Pd-109, Pm-149, Pr-142, Pr-143, Rb-82, Re-189, Rh-105, Sc-47, Se-75, Sr-83, Sr-89, Tb-161, Tc-94, Tc-99, Y-86, Y-90 and Zr-89.

33. The method of claim 1, further comprising administering a clearing composition to said tissue and allowing said clearing composition to

clear unbound said multi-specific antibody or antibody fragment from said tissue.

34. The method of claim 1, wherein said multi-specific antibody or antibody fragment is a monoclonal antibody.

35. The method of claim 1, wherein said multi-specific antibody or antibody fragment is **chimeric**, humanized, or human.

36. The method of claim 1, wherein said polymer is selected from the group consisting of polymers of single amino acids, co-polymers of two amino acids, co-polymers of three amino acids, co-polymers of four amino acids, polyethylene glycol (PEG), derivatives of PEG, co-polymers of PEG, N-(2-hydroxypropyl)methacrylamide (HPMA), polystyrene-co-maleic acid/anhydride (SMA), polyvinylether maleic anhydride (DIVEMA), polyethyleneimine, ethoxylated ployethyleneimine, starburst dendrimers, polyvinylpyrrolidone (PVP), apometallothionein and calicheamicin.

37. The method of claim 1, wherein said therapeutic agent is selected from the group consisting of a therapeutic radioisotope, toxin, cytokine, immunomodulator, drug, prodrug and boron addend.

38. The method of claim 37, wherein said drug is selected from the group consisting of taxanes, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes; folic acid analogs, pyrimidine analogs, purine analogs, vinca alkaloids, antibiotics, enzymes, platinum coordination complexes, substituted urea, methyl hydrazine derivatives, adrenocortical suppressants, antagonists, steroids, progestins, estrogens, antiestrogens, androgens, azaribine, anastrozole, azacytidine, bleomycin, bryostatin-1, busulfan, carmustine, chlorambucil, cisplatin, irinotecan (CPT-11), carbopiatin, celebrex, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, ethinyl estradiol, estramustine, etoposide, floxuridine, fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinblastine, vinorelbine or vincristine.

39. The method of claim 1, wherein said therapeutic or diagnostic agent is selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, contrasting agents, and coloring agents.

40. The method of claim 37, wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-α, interferon-β, interferon-γ, GM-CSF, G-CSF, erythropoietin and thrombopoietin.

41. A method for photodynamic diagnosis or treatment of a disease or disorder comprising: (a) administering to a tissue a multi-specific antibody or antibody fragment, comprising a targeting arm that binds to an antigen on said target site, and a capture arm that binds to a polymer conjugate; and (b) administering to said tissue a polymer conjugate that binds to said capture arm, said polymer conjugate comprising a polymer conjugated to a diagnostic or therapeutic agent.

42. The method of claim 41, wherein said therapeutic agent is a photosensitizer.

43. The method of claim 42, wherein said photosensitizer is selected from the group consisting of a dihematoporphyrin, benzoporphyrin monoacid ring A, tin etiopurpurin, sulfonated aluminum phthalocyanine, and lutetium texaphyrin.

44. An intravascular or endoscopic method for diagnosing or treating a disease or disorder comprising (a) administering to a tissue a multi-specific antibody or antibody fragment, comprising a targeting arm that binds to an antigen on said target site, and a capture arm that binds to a polymer conjugate; and (b) administering to said tissue a polymer conjugate that binds to said capture arm, said polymer conjugate comprising a polymer conjugated to a diagnostic or therapeutic agent.

45. The method of claim 44, wherein said disease or disorder is selected

from the group consisting of a cancer, cardiovascular lesion, an inflammatory disease, neurodegenerative disease, metabolic disease, and an infectious disease.

46. The method of claim 45, wherein said cancer is selected from the group consisting of a solid tumor, a B-cell malignancy and a T-cell malignancy.

47. The method of claim 46, wherein said disease or disorder is a B-cell malignancy selected from the group consisting of indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, and multiple myeloma.

48. The method of claim 46, wherein said solid tumor is selected from the group consisting of a melanoma, carcinoma, glioma and sarcoma.

49. The method of claim 48, wherein said carcinoma is selected from the group consisting of renal carcinoma, lung carcinoma, intestinal carcinoma, and stomach carcinoma.

50. The method of claim 45, wherein said cancer is selected from the group consisting of esophageal, gastric, colonic, rectal, pancreatic, lung, breast, ovarian, urinary bladder, endometrial, cervical, testicular, renal, adrenal and liver cancer.

51. The method of claim 45, wherein said cardiovascular lesion is selected from the group consisting of an infarct, clot, embolus, atherosclerotic plaque, and ischemia.

52. The method of claim 45, wherein said neurodegenerative disease is Alzheimer's disease.

53. The method of claim 45, wherein said metabolic disease is amyloidosis and said antibody binds amyloid.

54. The method of claim 44, wherein said disease or disorder is an autoimmune disease.

55. The method of claim 54, wherein said autoimmune disease is selected from the group consisting of myasthenia gravis, lupus nephritis, lupus erythematosus, and rheumatoid arthritis, Class III autoimmune diseases such as immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpura and chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sjogren's syndrome, multiple sclerosis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis ubiterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis and fibrosing alveolitis.

56. The method of claim 45, wherein said infectious disease is selected from the group consisting of a bacterial, fungal, parasitic and viral lesion.

57. The method of claim 56, wherein said infectious disease is caused by a fungus selected from the group consisting of Microsporum, Trichophyton, Epidermophyton, Ssporothrix schenckii, Cyrptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis, and Candida albicans.

58. The method of claim 56, wherein said infectious disease is caused by a virus selected from the group consisting of human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, **Dengue virus**, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus and blue tongue virus.

59. The method of claim 56, wherein said infectious disease is caused by a bacterium selected from the group consisting of Anthrax bacillus, Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis and Tetanus toxin.

60. The method of claim 56, wherein said infectious disease is caused by a parasite selected from the group consisting of a helminth or a malarial parasite.

61. The method of claim 45, wherein said infectious disease is caused by a protozoa selected from the group consisting of Plasmodium falciparum, Plasmodium vivax, Toxoplasma gondii, Trypanosoma rangeli, Trypanosoma cruzi, Trypanosoma rhodesiensei, Trypanosoma brucei, Schistosoma mansoni, Schistosoma japanicum, Babesia bovis, Elmeria tenella, Onchocerca volvulus, Leishmania tropica, Trichinella spiralis, Onchocerca volvulus, Theileria parva, Taenia hydatigena, Taenia ovis, Taenia saginata, Echinococcus granulosus and Mesocestoides corti.

62. The method of claim 45, wherein said infectious disease is caused by a mycoplasma selected from the group consisting of Mycoplasma arthritidis, Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma arginini, Acholeplasma laidlawii, Mycoplasma salivarum, and Mycoplasma pneumoniae.

63. The method of claim 45, wherein said antigen is selected from the group consisting of carcinoembryonic antigen (CEA), HER-2/neu, epidermal growth factor receptor (EGFR), VEGF, placental growth factor (PLGF), tenascin, EGP-1, EGP-2, CD19, CD20, CD22, CD21, CD23, CD30, CD33, CD45, CD80, and CD74. α-fetoprotein, A3, CA125, colon-specific antigen-p (CSAp), folate receptor, HLA-DR, human chorionic gonadrotropin, Ia, IL-2, insulin-like growth factor, KS-1, Le(y), MAGE, MUC1, MUC2, MUC3, MUC4, NCA66, necrosis antigens, PAM-4, prostatic acid phosphatase (PAP), Pr1, prostate specific antigen (PSA), PSMA, S100, T101, TAC, IL-6 and TAG-72.

64. The method of claim 45, wherein said cancer is selected from the group consisting of a CEA-expressing tumor or a CD20-expressing malignancy.

65. The method of claim 45, wherein said CD20-expressing malignancy is a B-cell lymphoma or leukemia.

66. The method of claim 44, wherein said polymer conjugate has a general formula comprising (polymer backbone)-$(agent)_m$, where m is an integer.

67. The method of claim 66, wherein said polymer conjugate further comprises a recognition hapten conjugated to said polymer.

68. The method of claim 44, wherein said polymer conjugate has a general formula comprising $(recognition\ hapten)_n$-(polymer backbone)-$(agent)_m$, where n and m are integers.

69. The method of claim 68, wherein said recognition hapten is selected from the group consisting of: diethylenetriaminepentaacetic acid (DTPA), a metal complex of DTPA, 1,4,7,10-tetrazacyclododecane-N,N',N",N'"-tetraacetic acid (DOTA), a metal complex of DOTA, N,N'-di[2-hydroxy-5-(ethylene-.E-backward.-carboxy)benzyl]ethylenediamine N,N'-diacetic acid (HBED), a metal complex of HBED, fluorescein, 2,4-dinitrophenyl-derivatives, biotin and histaminyl-succinyl-glycine.

70. The method of claim 44, further comprising administering a clearing composition to said tissue and allowing said clearing composition to clear unbound said multi-specific antibody or antibody fragment from said tissue.

71. The method of claim 44, wherein said multi-specific antibody or antibody fragment is a monoclonal antibody.

72. The method of claim 44, wherein said multi-specific antibody or antibody fragment is **chimeric**, humanized, or human.

73. The method of claim 44, wherein said polymer is selected from the group consisting of polymers of single amino acids, co-polymers of two amino acids, co-polymers of three amino acids, co-polymers of four amino acids, polyethylene glycol (PEG), derivatives of PEG, co-polymers of

PEG, N-(2-hydroxypropyl)methacrylamide (HPMA), polystyrene-co-maleic acid/anhydride (SMA), polyvinylether maleic anhydride (DIVEMA), polyethyleneimine, ethoxylated ploye thyleneimine, starburst dendrimers, polyvinylpyrrolidone (PVP), apometallothionein and calicheamicin.

L15 ANSWER 12 OF 22 USPATFULL on STN
2004:7312 Recombinant parainfluenza virus expression systems and vaccines comprising heterologous antigens derived from metapneumovirus.
Fouchier, Ronaldus Adrianus Maria, Rotterdam, NETHERLANDS
Van Den Hoogen, Bernadetta Gerarda, Rotterdam, NETHERLANDS
Osterhaus, Albertus Dominicus Marcellinus Erasmus, Bunnik, NETHERLANDS
Haller, Aurelia, Redwood City, CA, UNITED STATES
Tang, Roderick, San Carlos, CA, UNITED STATES
US 2004005545 A1 20040108
APPLICATION: US 2003-373567 A1 20030221 (10)
PRIORITY: US 2002-358934P 20020221 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to recombinant bovine parainfluenza virus (bPIV) cDNA or RNA which may be used to express heterologous gene products in appropriate host cell systems and/or to rescue negative strand RNA recombinant viruses that express, package, and/or present the heterologous gene product. In particular, the heterologous gene products include gene product of another species of PIV or from another negative strand RNA virus, including but not limited to, influenza virus, respiratory syncytial virus, human metapneumovirus and avian pneumovirus. The **chimeric** viruses and expression products may advantageously be used in vaccine formulations including vaccines against a broad range of pathogens and antigens.

CLM What is claimed is:

1. A **chimeric** bovine parainfluenza virus type 3 comprising a heterologous nucleotide sequence encoding a metapneumovirus polypeptide.

2. A **chimeric** human parainfluenza virus type 3 comprising a heterologous nucleotide sequence encoding a metapneumovirus polypeptide.

3. A **chimeric** bovine parainfluenza virus type 3/human parainfluenza virus type 3 comprising a heterologous nucleotide sequence encoding a metapneumovirus polypeptide.

4. The virus of claim 1, 2, or 3, wherein said virus comprises heterologous nucleotide sequences derived from the same gene of a metapneumovirus.

5. The virus of claim 1, 2, or 3, wherein said virus comprises heterologous nucleotide sequences derived from at least two different genes of a metapneumovirus.

6. The virus of claim 1, 2, or 3, wherein one or more of the open reading frames in the genome of the virus have been replaced by an ORF which encodes one or more of (i) an avian pneumovirus (APV) F protein; (ii) an APV G protein; (iii) an APV SH protein; (iv) an APV N protein; (v) an APV P protein; (vi) an APV M2 protein; (vii) an APV M2-1 protein; (viii) an APV M2-2 protein; or (ix) an APV L protein.

7. The virus of claim 1, 2, or 3, wherein one or more of the open reading frames in the genome of virus have been replaced by an ORF which encodes one or more of (i) a mammalian metapneumovirus (MPV) F protein; (ii) a mammalian MPV G protein; (iii) a mammalian MPV SH protein; (iv) a mammalian MPV N protein; (v) a mammalian MPV P protein; (vi) a mammalian MPV M2 protein; (vii) a mammalian MPV M2-1 protein; (viii) a mammalian MPV M2-2 protein; or (ix) a mammalian MPV L protein.

8. The virus of claim 6, wherein the avian pneumovirus is APV type A, APV type B, APV type C, or APV type D.

9. The virus of claim 7, wherein the mammalian MPV is variant A1, variant A2, variant B1, or variant B2.

10. The virus of claim 1, 2, or 3, wherein said heterologous nucleotide sequence is added to the complete genome of said virus.

11. The virus of claim 1, 2, or 3, wherein said nucleotide sequence is added at a nucleotide position of the parainfluenza virus genome of 104, 1774, and 3724.

12. The virus of claim 1, 2, or 3, wherein a nucleotide sequence of said parainfluenza virus has been substituted by said heterologous nucleotide

sequence.

13. The virus of claim 1, 2, or 3, wherein the heterologous sequences are inserted into the genome of said parainfluenza virus, wherein the genome has been deleted of one or more genes.

14. The virus of claim 1, wherein said bovine parainfluenza virus is a Kansas-strain bovine parainfluenza virus type 3.

15. The virus of claim 1, 2, or 3, wherein said heterologous nucleotide sequence is derived from the nucleotide sequences encoding a F protein, a G protein or a fragment thereof.

16. The virus of claim 15, wherein said F protein comprises an ectodomain of a F protein of a metapneumovirus, a transmembrane domain of a F protein of a parainfluenza virus, and luminal domain of a F protein of a parainfluenza virus.

17. The virus of claim 1, 2, or 3, wherein said nucleotide sequence is SEQ ID Nos: 1 to 5, 14, or 15.

18. The virus of claim 1, 2, or 3, wherein said nucleotide sequence encodes a protein of SEQ ID Nos: 6 to 13, 16, or 17.

19. The virus of claim 1, 2, or 3, wherein said virus further comprises a heterologous nucleotide sequence derived from a respiratory syncytial virus or a mutated form of respiratory syncytial virus.

20. The virus of claim 19, wherein the respiratory syncytial virus is a respiratory syncytial virus type A, a respiratory syncytial virus type B, a bovine respiratory syncytial virus, or an ovine respiratory syncytial virus.

21. The virus of claim 19, wherein said sequence is derived from the nucleotide sequences encoding a F protein, a G protein or a fragment thereof of said respiratory syncytial virus.

22. The **chimeric** parainfluenza virus of claim 1, 2, or 3, wherein the genome of said virus contains mutations or modifications, in addition to said heterologous nucleotide sequences, which result in a **chimeric** virus having a phenotype more suitable for use in vaccine formulations such as an attenuated phenotype or a phenotype with enhanced antigenicity.

23. The **chimeric** parainfluenza virus of claim 1, 2, or 3, wherein the intergenic region of said heterologous nucleotide sequence is altered.

24. The **chimeric** parainfluenza virus of claim 1, 2, or 3, wherein said heterologous nucleotide sequence is added at a position of the parainfluenza virus genome selected from the group consisting of position 1, 2, 3, 4, 5, and 6, and wherein the intergenic region of said heterologous nucleotide sequence is altered.

25. A recombinant DNA or RNA molecule encoding the genome of the virus of claim 1, 2, or 3.

26. A recombinant DNA or RNA molecule encoding the genome of the virus of claim 18.

27. A vaccine formulation comprising the **chimeric** virus of claim 1, 2, or 3, and a pharmaceutically acceptable excipient.

28. A vaccine formulation comprising the **chimeric** virus of claim 18 and a pharmaceutically acceptable excipient.

29. The vaccine formulation of claim 27, wherein said **chimeric** virus comprises a genomic modification or mutation which results in an attenuated phenotype or enhanced antigenicity.

30. The vaccine formulation of claim 29, wherein said modification or mutation is derived from a naturally occurring mutant.

31. The vaccine formulation of claim 27, wherein said vaccine is used to modulate the immune response of humans, primates, horses, cows, sheep, pigs, goats, dogs, cats, avian species or rodents.

32. The vaccine formulation of claim 30, wherein the vaccine is used to modulate the immune response of a human infant or a child.

33. An immunogenic formulation comprising the **chimeric** virus of claim 1, 2, or 3, and a pharmaceutically acceptable excipient.

34. An immunogenic formulation comprising the **chimeric** virus of claim 18 and a pharmaceutically acceptable excipient.

35. The immunogenic formulation of claim 33, wherein said **chimeric** virus comprises a genomic modification or mutation which results in an attenuated phenotype or enhanced antigenicity.

36. The immunogenic formulation of claim 35, wherein said modification or mutation is derived from a naturally occurring mutant.

37. The vaccine formulation of claim 33, wherein said vaccine is used to modulate the immune response of humans, primates, horses, cows, sheep, pigs, goats, dogs, cats, avian species or rodents.

38. The vaccine formulation of claim 36, wherein the vaccine is used to modulate the immune response of a human infant or a child.

39. A method for treating or preventing a respiratory tract infection in a mammal, said method comprising administering a vaccine of claim 27.

40. The method of claim 39, wherein the respiratory tract infection is a MPV infection.

41. The method of claim 39, wherein the respiratory tract infection is an infection with MPV, RSV, hPIV or a combination thereof.

42. The method of claim 39, wherein the subject is a human.

43. The method of claim 42, wherein the human subject is less than 5 years of age.

44. The method of claim 42, wherein the human subject is less than 2 years of age.

45. The method of claim 42, wherein the human subject suffers from a disease or a condition in addition to the respiratory tract infection.

46. The method of claim 45, wherein the disease or a condition is of cystic fibrosis, leukemia, non-Hodgkin lymphoma, asthma, and bone marrow transplantation and kidney transplantation.

47. The human subject of claim 42, wherein the human subject is an immunocompromised individual.

48. The human subject of claim 42, wherein the human subject is an elderly.

L15 ANSWER 13 OF 22 USPATFULL on STN
2004:7311 Metapneumovirus strains and their use in vaccine formulations and as vectors for expression of antigenic sequences.
Fouchier, Ronaldus Adrianus Maria, Rotterdam, NETHERLANDS
Van Den Hoogen, Bernadetta Gerarda, Rotterdam, NETHERLANDS
Osterhaus, Albertus Dominicus Marcellinus Erasmus, Bunnik, NETHERLANDS
Haller, Aurelia, Redwood City, CA, UNITED STATES
Tang, Roderick, San Carlos, CA, UNITED STATES
MedImmune Vaccines, Inc. (U.S. corporation)ViroNovative BV (U.S. corporation)
US 2004005544 A1 20040108
APPLICATION: US 2003-371122 A1 20030221 (10)
PRIORITY: EP 2001-203985 20011018
EP 2001-200213 20010119
WO 2002-NL40 20020118
US 2002-358934P 20020221 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides an isolated mammalian negative strand RNA virus, metapneumovirus (MPV), within the sub-family Pneumoviridae, of the family Paramyxoviridae. The invention also provides isolated mammalian negative strand RNA viruses identifiable as phylogenetically corresponding or relating to the genus Metapneumovirus and components thereof. In particular the invention provides a mammalian MPV, subgroups and variants thereof. The invention relates to genomic nucleotide sequences of different isolates of mammalian metapneumoviruses, in particular human metapneumoviruses. The invention relates to the use of the sequence information of different isolates of mammalian

metapneumoviruses for diagnostic and therapeutic methods. The present invention relates to nucleotide sequences encoding the genome of a metapneumovirus or a portion thereof, including both mammalian and avian metapneumovirus. The invention further encompasses **chimeric** or recombinant viruses encoded by said nucleotide sequences. The invention also relates to **chimeric** and recombinant mammalian MPV that comprise one or more non-native or heterologous sequences. The invention further relates to vaccine formulations comprising mammalian or avian metapneumovirus, including recombinant and **chimeric** forms of said viruses. The vaccine preparations of the invention encompass **multivalent** vaccines, including **bivalent** and trivalent vaccine preparations.

CLM What is claimed is:

1. An isolated negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus is a mammalian virus.

2. An isolated negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus is phylogenetically more closely related to a virus isolate deposited as I-2614 with CNCM, Paris than to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis.

3. An isolated negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus is phylogenetically more closely related to a virus isolate deposited as I-2614 with CNCM, Paris than related to APV type C.

4. An isolated negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus is phylogenetically more closely related to a virus isolate comprising the nucleotide sequence of SEQ ID NO:19 than it is related to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis.

5. An isolated negative-sense single stranded RNA metapneumovirus, wherein (i) the metapneumovirus belongs to the subfamily Pneumovirinae of the family Paramyxoviridae; and (ii) the metapneumovirus is identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus corresponds phylogenetically to the genus Metapneumovirus if the nucleic acid sequence of the virus in a phylogenetic tree analysis using 100 bootstraps and 3 jumbles is more closely related to a virus isolate deposited as I-2614 with CNCM, Paris than it is related to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis.

6. An isolated negative-sense single stranded RNA metapneumovirus, wherein (i) the metapneumovirus belongs to the subfamily Pneumovirinae of the family Paramyxoviridae; and (ii) the metapneumovirus is identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus corresponds phylogenetically to the genus Metapneumovirus if the nucleic acid sequence of the virus in a phylogenetic tree analysis using 100 bootstraps and 3 jumbles is more closely related to a virus isolate deposited as I-2614 with CNCM, Paris than it is related to APV type C.

7. An isolated negative-sense single stranded RNA metapneumovirus, wherein (i) the metapneumovirus belongs to the subfamily Pneumovirinae of the family Paramyxoviridae; and (ii) the metapneumovirus is identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus corresponds phylogenetically to the genus Metapneumovirus if the nucleic acid sequence of the virus in a phylogenetic tree analysis using 100 bootstraps and 3 jumbles is more closely related to a virus isolate comprising the nucleotide sequence of SEQ ID NO:18 than it is related to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis.

8. An isolated negative-sense single stranded RNA metapneumovirus, wherein the amino acid sequence of the N protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 90% identical to the amino acid sequence of SEQ ID NO:365, wherein sequence identity is determined over the entire length of the N protein.

9. An isolated negative-sense single stranded RNA metapneumovirus, wherein the amino acid sequence of the P protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 70% identical to the amino acid sequence of SEQ ID NO:373, wherein sequence identity is determined over the entire length of the P protein.

10. An isolated negative-sense single stranded RNA metapneumovirus, wherein the amino acid sequence of the M protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 90% identical to the amino acid sequence of SEQ ID NO:357, wherein sequence identity is determined over the entire length of the M protein.

11. An isolated negative-sense single stranded RNA metapneumovirus, wherein the amino acid sequence of the F protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 82% identical to the amino acid sequence of SEQ ID NO:314, wherein sequence identity is determined over the entire length of the F protein.

12. An isolated negative-sense single-stranded RNA metapneumovirus, wherein the amino acid sequence of the M2-1 protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 85% identical to the amino acid sequence of SEQ ID NO:337, wherein sequence identity is determined over the entire length of the M2-1 protein.

13. An isolated negative-sense single stranded RNA metapneumovirus, wherein the amino acid sequence of the M2-2 protein of the isolated negative-sense single stranded RNA metapneumovirus is at least 60% identical to the amino acid sequence of SEQ ID NO:345, wherein sequence identity is determined over the entire length of the M2-2 protein.

14. An isolated negative-sense single stranded RNA metapneumovirus, wherein the negative-sense single stranded RNA metapneumovirus encodes at least two proteins of the following: (i) a N protein with at least 90% amino acid sequence identity to SEQ ID NO:365; (ii) a P protein with at least 70% amino acid sequence identity to SEQ ID NO:373; (iii) a M protein with at least 90% amino acid sequence identity to SEQ ID NO:357; (iv) a F protein with at least 82% amino acid sequence identity to SEQ ID NO:314; (v) a M2-1 protein with at least 85% amino acid sequence identity to SEQ ID NO:337; or (vi) a M2-2 protein with at least 60% amino acid sequence identity to SEQ ID NO:345, wherein sequence identity is determined over the entire length of the respective protein.

15. An isolated negative-sense single stranded RNA metapneumovirus, wherein the negative-sense single stranded RNA metapneumovirus encodes: (i) a N protein with at least 90% amino acid sequence identity to SEQ ID NO:365; (ii) a P protein with at least 70% amino acid sequence identity to SEQ ID NO:373; (iii) a M protein with at least 90% amino acid sequence identity to SEQ ID NO:357; (iv) a F protein with at least 82% amino acid sequence identity to SEQ ID NO:314; (v) a M2-1 protein with at least 85% amino acid sequence identity to SEQ ID NO:337; or (vi) a M2-2 protein with at least 60% amino acid sequence identity to SEQ ID NO:345, wherein sequence identity is determined over the entire length of the respective protein.

16. An isolated negative-sense single stranded RNA metapneumovirus, wherein the genome of the virus comprises a nucleotide sequence of SEQ ID NO:19.

17. The virus of any one of claims 1-16, wherein the virus is an attenuated virus.

18. An immunogenic composition, wherein the immunogenic composition comprises the virus of any one of claims 1-16.

19. The immunogenic composition of claim 18, wherein the infectious recombinant virus is an attenuated virus.

20. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:365.

21. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 70% identical to SEQ ID NO:373.

22. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:357.

23. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 82% identical to SEQ ID NO:314.

24. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 85% identical to SEQ ID NO:337.

25. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 60% identical to SEQ ID NO:345.

26. An isolated nucleic acid, wherein the isolated nucleic acid hybridizes specifically under high stringency conditions with the nucleic acid of anyone of claims 20-25.

27. The isolated nucleic acid of claim 26, wherein said high stringency conditions comprise hybridization in a buffer consisting of 6×SSC, 50 mM Tris-HCl (pH=7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 100 µg/ml denatured salmon sperm DNA, for 48 hours at 65° C., washing in a buffer consisting of 2×SSC, 0.01% PVP, 0.01% Ficoll and 0.01% BSA, for 45 minutes at 37° C., and washing in a buffer consisting of 0.1×SSC, for 45 minutes at 50° C.

28. A method of detecting a mammalian metapneumovirus in a sample, wherein the method comprises contacting the sample with the nucleic acid of anyone of claims 20-25.

29. The method of claim 28, wherein the mammalian MPV is a human MPV.

30. A vector comprising the nucleic acid of anyone of claims 20-25.

31. A host cell comprising the nucleic acid of anyone of claims 20-25.

32. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising an amino acid sequence that is at least 90% identical to SEQ ID NO:365.

33. An isolated protein, wherein the protein comprises an amino acid sequence that is at least 70% identical to SEQ ID NO:373.

34. An isolated protein, wherein the protein comprises an amino acid sequence that is at least 90% identical to SEQ ID NO:357.

35. An isolated protein, wherein the protein comprises an amino acid sequence that is at least 82% identical to SEQ ID NO:314.

36. An isolated protein, wherein the protein comprises an amino acid sequence that is at least 85% identical to SEQ ID NO:337.

37. An isolated protein, wherein the protein comprises an amino acid sequence that is at least 60% identical to SEQ ID NO:345.

38. An antibody, wherein the antibody binds specifically to the protein of anyone of claims 33-37.

39. A method of detecting a mammalian metapneumovirus in a sample, wherein the method comprises contacting the sample with the antibody of claim 38.

40. A viral isolate, wherein the viral isolate is identifiable with a method according to claim 28 or 39 as a mammalian negative-sense single stranded RNA virus within the sub-family pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneunovirus.

41. A method for virologically diagnosing a MPV infection of a mammal comprising determining in a sample of said mammal the presence of a viral isolate or component thereof by contacting the sample with the nucleic acid of anyone of claims 20-25.

42. A method for serologically diagnosing a MPV infection in a mammal, wherein said method comprises detecting in a sample from the mammal the presence of an antibody specifically directed against an MPV or component thereof by reacting said sample with the protein of any one of claims 33-37.

43. A recombinant nucleic acid, wherein (i) the nucleic acid encodes

the genome of a mammalian negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus; and (ii) the nucleic acid further encodes a heterologous sequence.

44. An infectious **chimeric** virus, wherein the **chimeric** virus comprises (a) at least two contiguous kilobasebairs of nucleotide sequence derived from a mammalian MPV; and (b) a heterologous sequence.

45. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of a mammalian MPV, wherein one or more of the open reading frames in the genome of the mammalian MPV have been replaced by the analogous open reading frame from an avian MPV.

46. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of an avian MPV, wherein one or more of the open reading frames in the genome of the avian MPV have been replaced by the analogous open reading frame from mammalian MPV.

47. A pharmaceutical composition, wherein the pharmaceutical composition comprises (i) an isolated mammalian negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus Metapneumovirus, and (ii) a pharmaceutically acceptable carrier.

48. A pharmaceutical composition, wherein the pharmaceutical composition comprises (a) an isolated mammalian metapneumovirus, wherein (i) the metapneumovirus belongs to the subfamily Pneumovirinae of the family Paramyxoviridae; and (ii) the metapneumovirus is identifiable as phylogenetically corresponding to the genus Metapneumovirus, wherein the virus corresponds phylogenetically to the genus Metapneumovirus if the nucleic acid sequence of the virus in a phylogenetic tree analysis using 100 bootstraps and 3 jumbles is more closely related to a virus isolate deposited as I-2614 with CNCM, Paris than it is related to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis, and (b) a pharmaceutically acceptable carrier.

L15 ANSWER 14 OF 22 USPATFULL on STN
2003:330115 Metapneumovirus strains and their use in vaccine formulations and as vectors for expression of antigenic sequences.
Fouchier, Ronaldus Adrianus Maria, Rotterdam, NETHERLANDS
Van Den Hoogen, Bernadetta Gerarda, Rotterdam, NETHERLANDS
Osterhaus, Albertus Dominicus Marcellinus Erasmus, Bunnik, NETHERLANDS
Haller, Aurelia, Redwood City, CA, UNITED STATES
Tang, Roderick, San Carlos, CA, UNITED STATES
US 2003232326 A1 20031218
APPLICATION: US 2003-371099 A1 20030221 (10)
PRIORITY: US 2002-358934P 20020221 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides an isolated mammalian negative strand RNA virus, metapneumovirus (MPV), within the sub-family Pneumoviridae, of the family Paramyxoviridae. The invention also provides isolated mammalian negative strand RNA viruses identifiable as phylogenetically corresponding or relating to the genus Metapneumovirus and components thereof. In particular the invention provides a mammalian MPV, subgroups and variants thereof. The invention relates to genomic nucleotide sequences of different isolates of mammalian metapneumoviruses, in particular human metapneumoviruses. The invention relates to the use of the sequence information of different isolates of mammalian metapneumoviruses for diagnostic and therapeutic methods. The present invention relates to nucleotide sequences encoding the genome of a metapneumovirus or a portion thereof, including both mammalian and avian metapneumovirus. The invention further encompasses **chimeric** or recombinant viruses encoded by said nucleotide sequences. The invention also relates to **chimeric** and recombinant mammalian MPV that comprise one or more non-native or heterologous sequences. The invention further relates to vaccine formulations comprising mammalian or avian metapneumovirus, including recombinant and **chimeric** forms of said viruses. The vaccine preparations of the invention encompass **multivalent** vaccines, including **bivalent** and trivalent vaccine preparations.
CLM What is claimed is:
1. An isolated negative-sense single stranded RNA virus MPV belonging to the sub-family Pneumovirinae of the family Paramyxoviridae and identifiable as phylogenetically corresponding to the genus

Metapneumovirus, wherein the virus is phylogenetically more closely related to a virus isolate comprising the nucleotide sequence of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21 than it is related to turkey rhinotracheitis virus, the etiological agent of avian rhinotracheitis.

2. The isolated negative-sense single stranded RNA of claim 1, wherein the phylogenetic analysis uses 100 bootstraps and 3 jumbles.

3. An isolated negative-sense single stranded RNA metapneumovirus, wherein the genome of the virus comprises a nucleotide sequence of SEQ ID NO:18.

4. An isolated negative-sense single stranded RNA metapneumovirus, wherein the genome of the virus comprises a nucleotide sequence of SEQ ID NO:19.

5. An isolated negative-sense single stranded RNA metapneumovirus, wherein the genome of the virus comprises a nucleotide sequence of SEQ ID NO:20.

6. An isolated negative-sense single stranded RNA metapneumovirus, wherein the genome of the virus comprises a nucleotide sequence of SEQ ID NO:21.

7. An isolated nucleic acid, wherein the nucleic acid has a nucleotide sequence that is at least 70% identical to SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21, wherein sequence identity is determined over the entire length of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 or SEQ ID NO:22.

8. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B1 (SEQ ID NO:324); (ii) an amino acid sequence that is at least 98.5% identical to the N protein of a mammalian MPV variant Bi (SEQ ID NO:368); (iii) an amino acid sequence that is at least 96% identical the P protein of a mammalian MPV variant B1 (SEQ ID NO:376); (iv) an amino acid sequence that is identical the M protein of a mammalian MPV variant B1 (SEQ ID NO:360); (v) an amino acid sequence that is at least 99% identical the F protein of a mammalian MPV variant B1 (SEQ ID NO:316); (vi) an amino acid sequence that is at least 98% identical the M2-1 protein of a mammalian MPV variant B1 (SEQ ID NO:340); (vii) an amino acid sequence that is at least 99% identical the M2-2 protein of a mammalian MPV variant B1 (SEQ ID NO:348); (viii) an amino acid sequence that is at least 83% identical the SH protein of a mammalian MPV variant B1 (SEQ ID NO:384); or (ix) an amino acid sequence that is at least 99% identical the L protein a mammalian MPV variant B1 (SEQ i) NO:332).

9. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A1 (SEQ ID NO:322); (ii) an amino acid sequence that is at least 99.5% identical to the N protein of a mammalian MPV variant A1 (SEQ ID NO:366); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant A1 (SEQ ID NO:374); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A1 (SEQ ID NO:358); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A1 (SEQ ID NO:314); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein of a mammalian MPV variant A1 (SEQ ID NO:338); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A1 (SEQ ID NO:346); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A1 (SEQ ID NO:382); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a virus of a mammalian MPV variant A1 (SEQ ID NO:330).

10. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A2 (SEQ ID NO:332); (ii) an amino acid sequence that is at least 99.5% identical to the N protein of a mammalian MPV variant A2 (SEQ ID NO:367); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant A2 (SEQ ID NO:375); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A2 (SEQ ID NO:359); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A2 (SEQ ID NO:315); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein

of a mammalian MPV variant A2 (SEQ ID NO: 339); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A2 (SEQ ID NO:347); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A2 (SEQ ID NO:383); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant A2 (SEQ ID NO:331).

11. An isolated nucleic acid, wherein the nucleic acid encodes a protein comprising (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B2 (SEQ ID NO:325); (ii) an amino acid sequence that is at least 97% identical to the N protein of a mammalian MPV variant B2 (SEQ ID NO:369); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant B2 (SEQ ID NO:377); (iv) an amino acid sequence that is identical to the M protein of a mammalian MPV variant B2 (SEQ ID NO:361); (v) an amino acid sequence that is at least 99% identical to the F protein of a mammalian MPV variant B2 (SEQ ID NO:317); (vi) an amino acid sequence that is at least 98% identical to the M2-1 protein of a mammalian MPV variant B2 (SEQ ID NO:341); (vii) an amino acid sequence that is at least 99% identical to the M2-2 protein of a mammalian MPV variant B2 (SEQ ID NO:349); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant B2 (SEQ ID NO:385); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant B2 (SEQ ID NO:333).

12. An isolated nucleic acid, wherein the nucleic acid hybridizes specifically under high stringency conditions to the nucleic acid of claim 8, 9, 10 or 11.

13. An isolated nucleic acid, wherein the nucleic acid hybridizes under low stringency conditions to the nucleic acid of claim 8, 9, 10 or 11.

14. The isolated nucleic acid of claim 12, wherein said high stringency conditions comprise hybridization in a buffer consisting of 6×SSC, 50 mM Tris-HCl (pH=7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 100 µg/ml denatured salmon sperm DNA, for 48 hours at 65° C., washing in a buffer consisting of 2×SSC, 0.01% PVP, 0.01% Ficoll and 0.01% BSA, for 45 minutes at 37° C., and washing in a buffer consisting of 0.1×SSC, for 45 minutes at 50° C.

15. An isolated infectious virus, wherein the virus comprises the nucleic acid of claim 8, 9, 10 or 11.

16. A method for detecting a variant BI mammalian MPV in a sample, wherein the method comprises contacting the sample with the nucleic acid of claim 8.

17. A method for detecting a variant Al mammalian MPV in a sample, wherein the method comprises contacting the sample with the nucleic acid of claim 9.

18. A method for detecting a variant A2 mammalian MPV in a sample, wherein the method comprises contacting the sample with the nucleic acid of claim 10.

19. A method for detecting a variant B2 mammalian MPV in a sample, wherein the method comprises contacting the sample with the nucleic acid of claim 11.

20. An isolated protein, wherein the protein comprises: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B1 (SEQ ID NO:324); (ii) an amino acid sequence that is at least 98.5% identical to the N protein of a mammalian MPV variant B1 (SEQ ID NO:368); (iii) an amino acid sequence that is at least 96% identical the P protein of a mammalian MPV variant B1 (SEQ ID NO:376); (iv) an amino acid sequence that is identical the M protein of a mammalian MPV variant B1 (SEQ ID NO:360); (v) an amino acid sequence that is at least 99% identical the F protein of a mammalian MPV variant B1 (SEQ ID NO:316); (vi) an amino acid sequence that is at least 98% identical the M2-1 protein of a mammalian MPV variant B1 (SEQ ID NO:340); (vii) an amino acid sequence that is at least 99% identical the M2-2 protein of a mammalian MPV variant B1 (SEQ ID NO:348); (viii) an amino acid sequence that is at least 83% identical the SH protein of a mammalian MPV variant B1 (SEQ ID NO:384); or (ix) an amino acid sequence that is at least 99% identical the L protein a mammalian MPV variant B1 (SEQ ID NO:332).

21. An isolated protein, wherein the protein comprises: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A1 (SEQ ID NO:322); (ii) an amino acid sequence that is at least 99.5% identical to the N protein of a mammalian MPV variant A1 (SEQ ID NO:366); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant A1 (SEQ ID NO:374); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A1 (SEQ ID NO:358); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A1 (SEQ ID NO:314); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein of a mammalian MPV variant A1 (SEQ ID NO:338); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A1 (SEQ ID NO:346); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A1 (SEQ ID NO:382); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a virus of a mammalian MPV variant A1 (SEQ ID NO:330).

22. An isolated protein, wherein the protein comprises: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A2 (SEQ ID NO:332); (ii) an amino acid sequence that is at least 99.5% identical to the N protein of a mammalian MPV variant A2 (SEQ ID NO:367); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant A2 (SEQ ID NO:375); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A2 (SEQ ID NO:359); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A2 (SEQ ID NO:315); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein of a mammalian MPV variant A2 (SEQ ID NO: 339); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A2 (SEQ ID NO:347); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A2 (SEQ ID NO:383); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant A2 (SEQ ID NO:331).

23. An isolated protein, wherein the protein comprises: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B2 (SEQ ID NO:325); (ii) an amino acid sequence that is at least 97% identical to the N protein of a mammalian MPV variant B2 (SEQ ID NO:369); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant B2 (SEQ ifD NO:377); (iv) an amino acid sequence that is identical to the M protein of a mammalian MPV variant B2 (SEQ ID NO:361); (v) an amino acid sequence that is at least 99% identical to the F protein of a mammalian MPV variant B2 (SEQ ID NO:317); (vi) an amino acid sequence that is at least 98% identical to the M2-1 protein of a mammalian MPV variant B2 (SEQ ID NO:341); (vii) an amino acid sequence that is at least 99% identical to the M2-2 protein of a mammalian MPV variant B2 (SEQ ID NO:349); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant B2 (SEQ ID NO:385); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant B2 (SEQ ID NO:333).

24. An antibody, wherein the antibody binds specifically to a protein consisting of: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B1 (SEQ ID NO:324); (ii) an amino acid sequence that is at least 98.5% identical to the N protein of a mammalian MPV variant Bi (SEQ ID NO:368); (iii) an amino acid sequence that is at least 96% identical the P protein of a mammalian MPV variant B1 (SEQ ID NO:376); (iv) an amino acid sequence that is identical the M protein of a mammalian MPV variant B1 (SEQ ID NO:360); (v) an amino acid sequence that is at least 99% identical the F protein of a mammalian MPV variant B1 (SEQ ID NO:316); (vi) an amino acid sequence that is at least 98% identical the M2-1 protein of a mammalian MPV variant B1 (SEQ ID NO:340); (vii) an amino acid sequence that is at least 99% identical the M2-2 protein of a mammalian MPV variant B1 (SEQ ID NO:348); (viii) an amino acid sequence that is at least 83% identical the SH protein of a mammalian MPV variant B1 (SEQ ID NO:384); or (ix) an amino acid sequence that is at least 99% identical the L protein a mammalian MPV variant B1 (SEQ ID NO:332).

25. An antibody, wherein the antibody binds specifically to a protein consisting of: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A1 (SEQ ID NO:322); (ii) an amino acid sequence that is at least 99.5% identical to the N protein of a mammalian MPV variant A1 (SEQ ID NO:366); (iii) an amino acid sequence that is at least 96% identical to the P protein

of a mammalian MPV variant A1 (SEQ ID NO:374); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A1 (SEQ ID NO:358); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A1 (SEQ ID NO:314); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein of a mammalian MPV variant A1 (SEQ ID NO:338); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A1 (SEQ ID NO:346); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A1 (SEQ ID NO:382); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a virus of a mammalian MPV variant A1 (SEQ ID NO:330).

26. An antibody, wherein the antibody binds specifically to a protein consisting of: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant A2 (SEQ ID NO:332); (ii) an amino acid sequence that is at least 96% identical to the N protein of a mammalian MPV variant A2 (SEQ ID NO:367); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant A2 (SEQ ID NO:375); (iv) an amino acid sequence that is at least 99% identical to the M protein of a mammalian MPV variant A2 (SEQ ID NO:359); (v) an amino acid sequence that is at least 98% identical to the F protein of a mammalian MPV variant A2 (SEQ ID NO:315); (vi) an amino acid sequence that is at least 99% identical to the M2-1 protein of a mammalian MPV variant A2 (SEQ ID NO: 339); (vii) an amino acid sequence that is at least 96% identical to the M2-2 protein of a mammalian MPV variant A2 (SEQ ID NO:347); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant A2 (SEQ ID NO:383); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant A2 (SEQ ID NO:331).

27. An antibody, wherein the antibody binds specifically to a protein consisting of: (i) an amino acid sequence that is at least 66% identical to the G protein of a mammalian MPV variant B2 (SEQ ID NO:325); (ii) an amino acid sequence that is at least 97% identical to the N protein of a mammalian MPV variant B2 (SEQ ID NO:369); (iii) an amino acid sequence that is at least 96% identical to the P protein of a mammalian MPV variant B2 (SEQ ID NO:377); (iv) an amino acid sequence that is identical to the M protein of a mammalian MPV variant B2 (SEQ ID NO:361); (v) an amino acid sequence that is at least 99% identical to the F protein of a mammalian MPV variant B2 (SEQ ID NO:317); (vi) an amino acid sequence that is at least 98% identical to the M2-1 protein of a mammalian MPV variant B2 (SEQ ID NO:341); (vii) an amino acid sequence that is at least 99% identical to the M2-2 protein of a mammalian MPV variant B2 (SEQ ID NO:349); (viii) an amino acid sequence that is at least 84% identical to the SH protein of a mammalian MPV variant B2 (SEQ ID NO:385); or (ix) an amino acid sequence that is at least 99% identical to the L protein of a mammalian MPV variant B2 (SEQ ID NO:333).

28. A method for detecting a variant B1 mammalian MPV in a sample, wherein said method comprises contacting the sample with the antibody of claim 24.

29. A method for detecting a variant A1 mammalian MPV in a sample, wherein said method comprises contacting the sample with the antibody of claim 25.

30. A method for detecting a variant A2 mammalian MPV in a sample, wherein said method comprises contacting the sample with the antibody of claim 26.

31. A method for detecting a variant B2 mammalian MPV in a sample, wherein said method comprises contacting the sample with the antibody of claim 27.

32. A method for identifying a viral isolate as a mammalian MPV, wherein said method comprises contacting said isolate or a component thereof with the antibody of claim 24, 25, 26 or 27.

33. A method for virologically diagnosing a MPV infection of a mammal comprising determining in a sample of said mammal the presence of a viral isolate or component thereof by contacting the sample with the antibody of claim 24, 25, 26 or 27.

34. A method for virologically diagnosing a mammalian MPV infection of a subject, wherein said method comprises: (a) obtaining a sample from the subject; (b) contacting the sample with the antibody of claim 24, 25,

26 or 27, wherein if the antibody binds to the sample the subject is infected with mammalian MPV.

35. An infectious recombinant virus, wherein the recombinant virus comprises the genome of a mammalian MPV and further comprises a non-native MPV sequence.

36. A recombinant nucleic acid, wherein the recombinant nucleic acid comprises (i) a nucleic acid encoding a G polypeptide of an MPV A1 variant; and (ii) a nucleic acid encoding a non-native MPV polypeptide.

37. A recombinant nucleic acid, wherein the recombinant nucleic acid comprises (i) a nucleic acid encoding a G polypeptide of an MPV A2 variant; and (ii) a nucleic acid encoding a non-native MPV polypeptide.

38. A recombinant nucleic acid, wherein the recombinant nucleic acid comprises (i) a nucleic acid encoding a G polypeptide of an MPV B I variant; and (ii) a nucleic acid encoding a non-native MPV polypeptide.

39. A recombinant nucleic acid, wherein the recombinant nucleic acid comprises (i) a nucleic acid encoding a G polypeptide of an MPV B2 variant; and (ii) a nucleic acid encoding a non-native MPV polypeptide.

40. An isolated infectious recombinant virus, wherein the recombinant virus is encoded by the nucleic acid of claim 36, 37, 38 or 39.

41. The isolated infectious recombinant virus of claim 40, wherein the nucleic acid of claim 36, 37, 38 or 39 further comprises a heterologous sequence.

42. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of a mammalian MPV of a first variant, wherein one or more of the open reading frames in the genome of the mammalian MPV of the first variant have been replaced by the analogous open reading frame from a mammalian MPV of a second variant.

43. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of a mammalian MPV of a first variant, wherein one or more of open reading frames of a mammalian MPV of a second variant are inserted into the genome of the mammalian MPV of the first variant.

44. The infectious **chimeric** virus of claim 42 or 43, wherein (i) if the first variant is A1, the second variant is A2, B1 or B2; (ii) if the first variant is A2, the second variant is A1, B1 or B2; (iii) if the first variant is B1, the second variant is A1, A2 or B2; (iv) if the first variant is B2, the second variant is A1, A2, or B I.

45. The infectious **chimeric** virus of claim 42 or 43, wherein the analogous open reading frame encodes a F protein or a G protein.

46. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of a mammalian MPV, wherein one or more of the open reading frames in the genome of the mammalian MPV have been replaced by an ORF which encodes one or more of (i) an avian MPV F protein; (ii) an avian MPV G protein; (iii) an avian MPV SH protein; (iv) an avian MPV N protein; (v) an avian MPV P protein; (vi) an avian MPV M2 protein; (vii) an avian MPV M2-1 protein; (viii) an avian MPV M2-2 protein; or (ix) an avian MPV L protein.

47. An infectious **chimeric** virus, wherein the **chimeric** virus comprises the genome of an avian MPV, wherein one or more of the open reading frames in the genome of the avian MPV have been replaced by an ORF which encodes one or more of (i) a mammalian MPV F protein; (ii) a mammalian MPV G protein; (iii) a mammalian MPV SH protein; (iv) a mammalian MPV N protein; (v) a mammalian MPV P protein; (vi) a mammalian MPV M2 protein; (vii) a mammalian MPV M2-1 protein; (viii) a mammalian MPV M2-2 protein; or (ix) a mammalian MPV L protein.

48. The infectious **chimeric** virus of claim 35, 42, 43, 46 or 47, wherein the avian MPV is APV type A, APV type B, APV type C or APV type D.

49. The infectious **chimeric** virus of claim 35, 42, 43, 46 or 47, wherein the mammalian MPV is variant A1, variant A2, variant BI or variant B2.

50. The infectious virus of claim 35, 42, 43, 46 or 47, wherein the virus further comprises a heterologous nucleotide sequence.

51. The infectious virus of claim 50, wherein the heterologous nucleotide sequence is inserted at position 1, 2, 3, 4, 5, or 6 of the metapneumovirus genome.

52. The infectious virus of claim 50, wherein a nucleotide sequence of the genome of the virus is substituted with the heterologous nucleotide sequence.

53. The virus of claim 50, wherein the heterologous sequence is derived from a negative stand RNA virus.

54. The virus of claim 50, wherein the heterologous sequence is derived from a RSV, PIV, APV, or from a mammalian MPV.

55. The virus of claim 54, wherein the RSV is a respiratory syncytial virus type A, a respiratory syncytial virus type B, a bovine respiratory syncytial virus or an ovine respiratory syncytial virus.

56. The virus of claim 54, wherein said parainfluenza virus is a parainfluenza virus type 1, a parainfluenza virus type 2, a parainfluenza virus type 3, a parainfluenza virus type 4 or a bovine parainfluenza virus.

57. The virus of anyone of claims 50-56, wherein the heterologous sequence encodes a F protein or a G protein.

58. The virus of claim 50, wherein said heterologous sequence is derived from a virus that causes Acquired Immune Deficiency Syndrome.

59. The virus of claim 35, 40, 42, 43, 46, or 47, wherein the virus is an attenuated virus.

60. The virus of claim 35, 40, 42, 43, 46, or 47, wherein the genome of said virus contains mutations or modifications, in addition to said heterologous nucleotide sequences, that result in a **chimeric** virus having a phenotype more suitable for use in vaccine formulations such an attenuated phenotype or a phenotype with enhanced antigenicity or enhanced immunogenicity.

61. The virus of claim 35, 40, 42, 43, 46, or 47, wherein the genome of the mammalian MPV is a mammalian MPV comprising the nucleotide sequence of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21.

62. An immunogenic composition, wherein the immunogenic composition comprises the infectious recombinant virus of claim 35, 40, 42, 43, 46, or 47.

63. A pharmaceutical composition, wherein the pharmaceutical composition comprises the infectious recombinant virus of claim 35, 40, 42, 43, 46, or 47.

64. A method for treating or preventing a respiratory tract infection in a mammal, said method comprising administering a vaccine comprising a mammalian metapneumovirus.

65. A method for treating or preventing a respiratory tract infection in a mammal, said method comprising administering a vaccine comprising the recombinant mammalian metapneumovirus of claim 35, 40, 42, 43, 46, or 47.

66. A method for treating or preventing a respiratory tract infection in a mammal, said method comprising administering a vaccine comprising avian metapneumovirus.

67. A method for treating or preventing a respiratory tract infection in a human, said method comprising administering a vaccine comprising avian metapneumovirus.

68. A method for treating or preventing a respiratory tract infection in a subject, said method comprising administering to the subject the composition of claim 62 or 63.

69. The method of claim 64, 65, 66, 67 or 68, wherein the respiratory tract infection is a MPV infection.

70. The method of claim 64, 65, 66, 67 or 68, wherein the respiratory tract infection is an infection with MPV and RSV.

71. The method of claim 64, 65, 66, 67 or 68, wherein the subject is a

human.

72. The method of claim 71, wherein the human subject is less than 5 years of age.

73. The method of claim 71, wherein the human subject is less than 2 years of age.

74. The method of claim 71, wherein the human subject suffers from a disease or a condition in addition to the respiratory tract infection.

75. The method of claim 71, wherein the disease or condition is selected from a group consisting of cystic fibrosis, leukaemia, non-Hodgkin lymphoma, Asthma, and bone marrow transplantation and kidney transplantation.

76. The method of claim 71, wherein the human subject is an immunocompromised individual.

77. The method of claim 71, wherein the human subject is an elderly.

78. A method for identifying a compound useful for the treatment of infections with mammalian MPV, wherein the method comprises: (a) infecting an animal with a mammalian MPV; (b) administering to the animal a test compound; and (c) determining the effect of the test compound on the infection of the animal, wherein a test compound that reduces the extent of the infection or that ameliorates the symptoms associated with the infection is identified as a compound useful for the treatment of infections with mammalian MPV.

79. A method for identifying a compound useful for the treatment of infections with mammalian MPV, wherein the method comprises: (a) infecting a cell culture with a mammalian MPV; (b) incubating the cell culture with a test compound; and (c) determining the effect of the test compound on the infection of the cell culture, wherein a test compound that reduces the extent of the infection is identified as a compound useful for the treatment of infections with mammalian MPV.

80. A method for diagnosing a mammalian MPV infection of an animal, wherein the method comprises determining in a sample of said animal the presence of a viral isolate or component thereof by reacting said sample with a nucleic acid or an antibody reactive with a component of an aviant pneumovirus, said nucleic acid or antibody being cross-reactive with a component of MPV.

81. A method for serologically diagnosing a mammalian MPV infection of an animal, wherein the method comprises contacting a sample from the animal with the protein of claim 20, 21, 22 or 23.

82. A method for serologically diagnosing a mammalian MPV infection of an animal, wherein the method comprises contacting a sample from the animal with a protein of an APV.

83. A method for diagnosing an APV infection of a bird comprising contacting a sample from the animal with the protein of claim 20, 21, 22 or 23.

84. The method of claim 83, wherein said APV is APV-C.

L15 ANSWER 15 OF 22 USPATFULL on STN
2003:329850 Recombinant parainfluenza virus expression systems and vaccines comprising heterologous antigens derived from metapneumovirus.
Fouchier, Ronaldus Adrianus Maria, Rotterdam, NETHERLANDS
Van Den Hoogen, Bernadetta Gerarda, Rotterdam, NETHERLANDS
Osterhaus, Albertus Dominicus Marcellinus Erasmus, Bunnik, NETHERLANDS
Haller, Aurelia, Redwood City, CA, UNITED STATES
Tang, Roderick, San Carlos, CA, UNITED STATES
US 2003232061 A1 20031218
APPLICATION: US 2003-371264 A1 20030221 (10)
PRIORITY: EP 2001-203985 20011018
WO 2002-NL40 20020118
US 2002-358934P 20020221 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to recombinant bovine parainfluenza virus (bPIV) cDNA or RNA which may be used to express heterologous gene products in appropriate host cell systems and/or to rescue negative strand RNA recombinant viruses that express, package, and/or present the

heterologous gene product. In particular, the heterologous gene products include gene product of another species of PIV or from another negative strand RNA virus, including but not limited to, influenza virus, respiratory syncytial virus, human metapneumovirus and avian pneumovirus. The **chimeric** viruses and expression products may advantageously be used in vaccine formulations including vaccines against a broad range of pathogens and antigens.

CLM What is claimed is:

1. A **chimeric** parainfluenza virus type 3 comprising a heterologous nucleotide sequence encoding a metapneumovirus polypeptide.

2. The virus of claim 1 wherein the heterologous nucleotide sequence is derived from a human metapneumovirus.

3. The virus of claim 1 wherein the heterologous sequence is derived from a mammalian metapneumovirus.

4. The virus of claim 1 wherein the heterologous sequence is derived from an avian pneumovirus.

5. The virus of claim 2, 3 or 4 wherein the heterologous sequence is derived from a F protein, a G protein, a SH protein, a N protein, a P protein, a M2 protein, a M2-1 protein, a M2-2 protein, or a L protein.

6. A recombinant DNA or RNA molecule encoding the genome of the virus of claim 1, 2, 3, or 4.

7. A recombinant DNA or RNA molecule encoding the genome of the virus of claim 5.

8. A vaccine formulation comprising the **chimeric** virus of claim 1, 2, 3, or 4 and a pharmaceutically acceptable excipient.

9. A vaccine formulation comprising the **chimeric** virus of claim 5 and a pharmaceutically acceptable excipient.

10. A method of treating a respiratory tract infection in a mammal, said method comprising administering the vaccine of claim 8.

11. A method of treating a respiratory tract infection in a mammal, said method comprising administering the vaccine of claim 9.

12. The method of claim 10 wherein the mammal is a human.

13. The method of claim 11 wherein the mammal is a human.

L15 ANSWER 16 OF 22 USPATFULL on STN

2003:300247 CD4-IgG2-based salvage therapy of HIV-1 infection.

Olson, William C., Ossining, NY, UNITED STATES
Israel, Robert J., Suffern, NY, UNITED STATES

US 2003211470 A1 20031113

APPLICATION: US 2003-386763 A1 20030312 (10)

PRIORITY: US 2002-413725P 20020926 (60)
US 2002-393249P 20020701 (60)
US 2002-364515P 20020315 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides the CD4-IgG2 **chimeric** heterotetramer, wherein the heavy chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193). This invention also provides the CD4-IgG2 **chimeric** heterotetramer, wherein the light chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194). This invention also provides the CD4-IgG2 **chimeric** heterotetramer, wherein the heavy chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and the light chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194). Finally, this invention provides a method of inhibiting HIV infection of a CD4+ cell, a method of preventing a subject from being infected with HIV, and a method of treating a subject infected with HIV so as to block the spread of HIV infection, using the above CD-4-IgG2 **chimeric** heterotetramers.

CLM What is claimed is:

1. A method of inhibiting HIV-1 infection of a CD4+ cell in an HIV-1-infected subject, which method comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to inhibit the infection by HIV-1 of uninfected CD4+ cells in said subject,

wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, each said heavy chain having the amino acid sequence set forth in FIGS. 4A-4H and each said light chain having the amino acid sequence set forth in FIGS. 5A-5D, and wherein said subject, prior to said administration, has at least one of (a) a low CD4+ cell count and (b) a high HIV-1 load.

2. The method of claim 1, wherein each said heavy chain is encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

3. The method of claim 1, wherein each said light chain is encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

4. The method of claim 1, wherein the subject has a low CD4+ cell count.

5. The method of claim 1, wherein the subject has a high HIV-1 load.

6. The method of claim 1, wherein the subject has both a low CD4+ cell count and a high HIV-1 load.

7. The method of claim 1, where the subject is a human.

8. The method of claim 1, wherein the CD4-IgG2 **chimeric** heterotetramer is administered to said subject in a dosage of from about 1 mg/kg to about 25 mg/kg per body weight of said subject.

9. The method of claim 1, wherein the **chimeric** heterotetramer is bound to a toxin.

10. The method of claim 9, wherein the toxin is selected from the group consisting of a deglycosylated A chain of ricin, domain II of pseudomonas exotoxin A, domain III of pseudomonas exotoxin A, and diphtheria toxin.

11. The method of claim 1, wherein the **chimeric** heterotetramer is bound to a detectable marker.

12. The method of claim 11, wherein the detectable marker is selected from the group consisting of a radioisotope, a chromophore and a fluorophore.

13. The method of claim 1, wherein prior to administration the HIV-1 of the subject has resistance to members of two classes of anti-retroviral agents.

14. The method of claim 1, wherein prior to administration the HIV-1 of the subject has resistance to multiple anti-retroviral agents.

15. The method of claim 1, wherein prior to administration the HIV-1 of the subject demonstrates sensitivity to the heterotetramer in vitro.

16. The method of claim 15, wherein the sensitivity is determined using an HIV-1 entry assay.

17. A method of reducing the amount of HIV-1 present in the CD4+ cells of an HIV-1-infected subject, which method comprises administering to the subject an amount of a CD4-TgG2 **chimeric** heterotetramer effective to reduce the amount of HIV-1 present in the subject's CD4+ cells, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, each said heavy chain having the amino acid sequence set forth in FIGS. 4A-4H and each said light chain having the amino acid sequence set forth in FIGS. 5A-5D, and wherein the subject, prior to said administration, has at least one of (a) a low CD4+ cell count and (b) a high HIV-1 load.

18. The method of claim 17, wherein prior to administration the HIV-1 of the subject has resistance to members of two classes of anti-retroviral agents.

19. The method of claim 17, wherein prior to administration the HIV-1 of the subject has resistance to multiple anti-retroviral agents.

20. The method of claim 17, wherein prior to administration the HIV-1 of the subject demonstrates sensitivity to the heterotetramer in vitro.

21. The method of claim 20, wherein the sensitivity is determined using an HIV-1 entry assay.

22. The method of claim 17, wherein each said heavy chain is encoded by

an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

23. The method of claim 17, wherein each said light chain is encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

24. The method of claim 17, wherein the subject has a low CD4+ cell count.

25. The method of claim 17, wherein the subject has a high HIV-1 load.

26. The method of claim 17, wherein the subject has both a low CD4+ cell count and a high HIV-1 load.

27. The method of claim 17, wherein the subject is a human.

28. The method of claim 17, wherein the CD4-IgG2 **chimeric** heterotetramer is administered to said subject in a dosage of from about 5 mg/kg to about 25 mg/kg per body weight of said subject.

29. The method of claim 17, wherein the **chimeric** heterotetramer is bound to a toxin.

30. The method of claim 29, wherein the toxin is selected from the group consisting of a deglycosylated A chain of ricin, domain II of pseudomonas exotoxin A, domain III of pseudomonas exotoxin A, and diphtheria toxin.

31. The method of claim 0.17, wherein the **chimeric** heterotetramer is bound to a detectable marker.

32. The method of claim 31, wherein the detectable marker is selected from the group consisting of a radioisotope, a chromophore and a fluorophore.

33. A method for reducing the amount of HIV-1 present within an HIV-1-infected subject, which method comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to reduce the amount of HIV-1 in said subject, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, each said heavy chain having the amino acid sequence set forth in FIGS. 4A-4H and each said light chain having the amino acid sequence set forth in FIGS. 5A-5D, and wherein the subject, prior to said administration, has at least one of (a) a low CD4+ cell count and (b) a high HIV-1 load.

34. The method of claim 33, wherein prior to administration the HIV-1 of the subject has resistance to members of two classes of anti-retroviral agents.

35. The method of claim 33, wherein prior to administration the HIV-1 of the subject has resistance to multiple anti-retroviral agents.

36. The method of claim 33, wherein prior to administration the HIV-1 the subject demonstrates sensitivity to the heterotetramer in vitro.

37. The method of claims 36, wherein the sensitivity is determined using an HIV-1 entry assay.

38. The method of claim 33, wherein each said heavy chain is encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

39. The method of claim 33, wherein each said light chain is encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

40. The method of claim 33, wherein the subject has a low CD4+ cell count.

41. The method of claim 33, wherein the subject has a high HIV-1 load.

42. The method of claim 33, wherein the subject has both a low CD4+ cell count and a high HIV-1 load.

43. The method of claim 33, wherein the subject is a human.

44. The method of claim 33, wherein the CD4-IgG2 **chimeric** heterotetramer is administered to said subject in a dosage of from about 5 mg/kg to about 25 mg/kg per body weight of said subject.

45. The method of claim 33, wherein the **chimeric** heterotetramer is

bound to a toxin.

46. The method of claim 45, wherein the toxin is selected from the group consisting of a deglycosylated chain A of ricin, domain II of pseudomonas exotoxin A, domain III of psudomonas exotoxin A, and diphtheria toxin.

47. The method of claim 33, wherein the **chimeric** heterotetramer is bound to a detectable marker.

48. The method of claim 47, wherein the detectable marker is selected from the group consisting of a radioisotope, a chromophore and a fluorophore.

49. A method of treating an HIV-1-infected subject, which method comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to treat said subject, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, each said heavy chain having the amino acid sequence set forth in FIGS. 4A-4H and each light chain having the amino acid sequence set forth in FIGS. 5A-5D, and wherein the subject, prior to said administration, has at least one of (a) a low CD4+ cell count and (b) a high HIV-1 load.

50. The method of claim 49, wherein prior to administration the HIV-1 of the subject has resistance to members of two classes of anti-retroviral agents.

51. The method of claim 49, wherein prior to administration the HIV-1 of the subject has resistance to multiple anti-retroviral agents.

52. The method of claim 49, wherein prior to administration the HIV-1 of the subject demonstrates sensitivity to the heterotetramer in vitro.

53. The method of claim 52, wherein the sensitivity is determined using an HIV-1 entry assay.

54. The method of claim 49, wherein each said heavy chain is encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

55. The method of claim 49, wherein each said light chain is encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

56. The method of claim 49, wherein the subject has a low CD4+ cell count.

57. The method of claim 49, wherein the subject has a high HIV-1 load.

58. The method of claim 49, wherein the subject has both a low CD4+ cell count and a high HIV-1 load.

59. The method of claim 49, wherein the subject is a human.

60. The method of claim 49, wherein the CD4-IgG2 **chimeric** heterotetramer is administered to said subject in a dosage of from about 1 mg/kg to about 25 mg/kg per body weight of said subject.

61. The method of claim 49, wherein the **chimeric** heterotetramer is bound to a toxin.

62. The method of claim 61, wherein the toxin is selected from the group consisting of a deglycosylated A chain of ricin, domain II of pseudomonas exotoxin A, domain III of pseudomonas exotoxin A, and diphtheria toxin.

63. The method of claim 49, wherein the **chimeric** heterotetramer is bound to a detectable marker.

64. The method of claim 63, wherein the detectable marker is selected from the group consisting of a radioisotope, a chromophore and a fluorophore.

65. A method of inhibiting or reducing HIV-1 viral load in an HIV-1-infected subject, which method comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to inhibit or to reduce the HIV-1 viral load in said HIV-1-infected subject, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, each said heavy chain having the amino acid sequence set forth in FIGS. 4A-4H and each said light chain

having the amino acid sequence set forth in FIGS. 5A-5D, and wherein said subject, prior to said administration, has at least one of (a) a low CD4+ cell count and (b) a high HIV-1 load.

66. The method of claim 65, wherein prior to administration the HIV-1 of the subject has resistance to members of two classes of anti-retroviral agents.

67. The method of claim 65, wherein prior to administration the HIV-1 of the subject has resistance to multiple anti-retroviral agents.

68. The method of claim 65, wherein prior to administration the HIV-1 of the subject demonstrates sensitivity to the heterotetramer in vitro.

69. The method of claim 68, wherein the sensitivity is determined using an HIV-1 entry assay.

70. The method of claim 65, wherein each said heavy chain is encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

71. The method of claim 65, wherein each said light chain is encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

72. The method of claim 65, wherein the subject has a low CD4+ cell count.

73. The method of claim 65, wherein the subject has a high HIV-1 load.

74. The method of claim 65, wherein the subject has both a low CD4+ cell count and a high HIV-1 load.

75. The method of claim 65, wherein the subject is a human.

76. The method of claim 65, wherein the CD4-IgG2 **chimeric** heterotetramer is administered to said subject in a dosage of from about 1 mg/kg to about 25 mg/kg per body weight of said subject.

77. The method of claim 65, wherein the **chimeric** heterotetramer is bound to a toxin.

78. The method of claim 77, wherein the toxin is selected from the group consisting of a deglycosylated A chain of ricin, domain II of pseudomonas exotoxin A, domain III of pseudomonas exotoxin A and diphtheria toxin.

79. The method of claim 65, wherein the **chimeric** heterotetramer is bound to a detectable marker.

80. The method of claim 79, wherein the detectable marker is selected from the group consisting of a radioisotope, a chromophore and a fluorophore.

81. The method of claim 65, wherein the method provides a viral load reduction of at least about 0.4 log10 copies/ml.

82. The method of claim 65, wherein the method provides a viral load reduction of at least about 0.8 log10 copies/ml.

83. The method of claim 65, wherein the method provides a viral load reduction of from about 0.4 to about 0.8 log10 copies/ml.

84. The method of claim 65, wherein the method provides an increase in the subject's CD4+ count.

L15 ANSWER 17 OF 22 USPATFULL on STN
2002:280145 Use of recombinant parainfluenza viruses (PIVs) as vectors to protect against infection and disease caused by PIV and other human pathogens.
Murphy, Brian R., Bethesda, MD, UNITED STATES
Collins, Peter L., Rockville, MD, UNITED STATES
Schmidt, Alexander C., Washington, DC, UNITED STATES
Durbin, Anna P., Takoma Park, MD, UNITED STATES
Skiadopoulos, Mario H., Potomac, MD, UNITED STATES
Tao, Tao, Bethesda, MD, UNITED STATES
US 2002155581 A1 20021024
APPLICATION: US 2000-733692 A1 20001208 (9)
PRIORITY: US 1997-47575P 19970523 (60)
US 1997-59385P 19970919 (60)

US 1999-170195P 19991210 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB **Chimeric** parainfluenza viruses (PIVs) are provided that incorporate a PIV vector genome or antigenome and one or more antigenic determinant(s) of a heterologous PIV or non-PIV pathogen. These **chimeric** viruses are infectious and attenuated in humans and other mammals and are useful in vaccine formulations for eliciting an immune responses against one or more PIVs, or against a PIV and non-PIV pathogen. Also provided are isolated polynucleotide molecules and vectors incorporating a **chimeric** PIV genome or antigenome which includes a partial or complete PIV vector genome or antigenome combined or integrated with one or more heterologous gene(s) or genome segment(s) encoding antigenic determinant(s) of a heterologous PIV or non-PIV pathogen. In preferred aspects of the invention, **chimeric** PIV incorporate a partial or complete human, bovine, or human-bovine **chimeric**, PIV vector genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) from a heterologous PIV or non-PIV pathogen, wherein the **chimeric** virus is attenuated for use as a vaccine agent by any of a variety of mutations and nucleotide modifications introduced into the **chimeric** genome or antigenome.

CLM What is claimed is:

1. An isolated infectious **chimeric** parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete PIV vector genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a **chimeric** PIV genome or antigenome.

2. The **chimeric** PIV of claim 1, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of the partial or complete PIV vector genome or antigenome.

3. The **chimeric** PIV of claim 1, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV vector genome or antigenome.

4. The **chimeric** PIV of claim 1, wherein said one or more heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or fragment(s) thereof.

5. The **chimeric** PIV of claim 1, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more heterologous PIV(s).

6. The **chimeric** PIV of claim 5, wherein said one or more heterologous PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

7. The **chimeric** PIV of claim 5, wherein the vector genome or antigenome is a partial or complete HPIV genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more heterologous HPIV(s).

8. The **chimeric** PIV of claim 7, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more beterologous HPIV(s).

9. The **chimeric** PIV of claim 8, wherein one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from HPIV1 HN and F **glycoproteins** and antigenic domains, fragments and epitopes thereof is/are added to or substituted within the partial or complete HPIV3 genome or antigenome.

10. The **chimeric** PIV of claim 8, wherein the vector genome or antigenome is a partial or complete HPIV3 JS genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more heterologous HPIV(s).

11. The **chimeric** PIV of claim 10, wherein one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from HPIV1 HN and F **glycoproteins** and antigenic domains, fragments and epitopes thereof is/are added to or substituted within the

partial or complete HPIV3 JS genome or antigenome.

12. The **chimeric** PIV of claim 9, wherein both HPIV1 genes encoding HN and F **glycoproteins** are substituted for counterpart HPIV3 HN and F genes in a partial HPIV3 vector genome or antigenome.

13. The **chimeric** PIV of claim 9, wherein the **chimeric** genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS cp45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position corresponding to Ile96 of JS cp45, a nucleotide substitution a 3' leader sequence of the **chimeric** virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45

14. The **chimeric** PIV of claim 8, wherein one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HPIV2 is/are added to or incorporated within the partial or complete HPIV3 genome or antigenome.

15. The **chimeric** PIV of claim 14, wherein one or more HPIV2 gene(s) or genome segment(s) encoding one or more HN and/or F **glycoprotein**(s) or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete HPIV3 vector genome or antigenome.

16. The **chimeric** PIV of claim 6, wherein a plurality of heterologous genes or genome segments encoding antigenic determinants of multiple heterologous PIVs are added to or incorporated within the partial or complete HPIV vector genome or antigenome.

17. The **chimeric** PIV of claim 16, wherein said plurality of heterologous genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 are added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

18. The **chimeric** PIV of claim 17, wherein one or more HPIV1 gene(s) or genome segment(s) encoding one or more HN and/or F **glycoprotein**(s) or antigenic domain(s), fragment(s) or epitope(s) thereof and one or more HPIV2 gene(s) or genome segment(s) encoding one or more HN and/or F **glycoprotein**(s) or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete HPIV3 vector genome or antigenome.

19. The **chimeric** PIV of claim 18, wherein both HPIV1 genes encoding HN and F **glycoproteins** are substituted for counterpart HPIV3 HN and F genes to form a **chimeric** HPIV3-1 vector genome or antigenome which is further modified by addition or incorporation of one or more gene(s) or gene segment(s) encoding one or more antigenic determinant(s) of HPIV2.

20. The **chimeric** PIV of claim 19, wherein a transcription unit comprising an open reading frame (ORF) of an HPIV2 RN gene is added to or incorporated within the **chimeric** HPIV3-1 vector genome or antigenome.

21. The **chimeric** PIV of claim 20 selected from rPIV3-1.2HN, or rPIV3-1cp45.2HN.

22. The **chimeric** PIV of claim 1, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

23. The **chimeric** PIV of claim 22, wherein said one or more heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F proteins, human papilloma virus LI protein, type 1 or type 2 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus pre M, E, and NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

24. The **chimeric** PIV of claim 22, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome or a **chimeric** HPIV genome or antigenome comprising a partial or complete HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous HPIV added or incorporated therein.

25. The **chimeric** PIV of claim 24, wherein the heterologous pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

26. The **chimeric** PIV of claim 25, wherein a transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within a HPIV3 vector genome or antigenome.

27. The **chimeric** PIV of claim 26 selected from rPIV3 (HA HN-L), rPIV3 (HA N-P), rcp45L(HA N-P), rPIV3 (HA P-M), or rcp45L(HA P-M).

28. The **chimeric** PIV of claim 24, wherein the vector genome or antigenome is a **chimeric** HPIV genome or antigenome comprising a partial or complete HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 added or incorporated therein.

29. The **chimeric** PIV of claim 25, wherein the heterologous pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

30. The **chimeric** PIV of claim 29, wherein a transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within a HPIV3-1 vector genome or antigenome having both the HPIV3 HN and F ORFs substituted by the HN and F ORFs of HPIV 1.

31. The **chimeric** PIV of claim 30, selected from rPIV3-1 HA_{P-M} or rPIV3-1 HA_{P-M} cp45L.

32. The **chimeric** PIV of claim 1, wherein the partial or complete PIV vector genome or antigenome is combined with one or more supernumerary heterologous gene(s) or genome segment(s) to form the **chimeric** PIV genome or antigenome.

33. The **chimeric** PIV of claim 32, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome and said one or more supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV 1 HN, HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene unit.

34. The **chimeric** PIV of claim 33, wherein one or both of the HPIV1 HN and/or HPIV2 HN ORF(s) is/are inserted within the HPIV3 vector genome or antigenome, respectively.

35. The **chimeric** PIV of claim 33, wherein the HPIV1 HN, HPIV2 HN, and measles virus HA ORFs are inserted between the N/P, P/M, and HN/L genes, respectively.

36. The **chimeric** PIV of claim 33, wherein the HPIV1 HN and HPIV2 HN genes are inserted between the N/P and P/M genes, respectively and a 3918-nt GU insert is added between the HN and L genes.

37. The **chimeric** PIV of claim 33, which is selected from rHPIV3 ^{1HNN} P, rHPIV3 1HNP-M, rHPIV3 $2HN_{N-P}$, rHPIV3 $2HN_{P-M}$, rHPIV3 $1HN_{N-P}$ $2HN_{P-M}$, rHPIV3 $1HN_{N-P}$ $^{2HN}{}_{P-M}$ HA_{HN-L} and rHPIV3 $1HN_{N-P}$ $2HN_{P-M}$ $3918GU_{HN-L}$.

38. The **chimeric** PIV of claim 32, which contains protective antigens from one, two, three or four pathogens.

39. The **chimeric** PIV of claim 32, which contains protective antigens from one to four pathogens selected from HPIV3, HPIV1, HPIV2, and measles virus.

40. The **chimeric** PIV of claim 32, wherein said one or more supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to the wild-type HPIV3 genome length of

15,462 nt.

41. The **chimeric** PIV of claim 32, wherein the addition of said one or more supernumerary heterologous gene(s) or genome segment(s) specifies an attenuation phenotype of the **chimeric** PIV which exhibits at least a 10-to 100-fold decrease in replication in the upper and/or lower respiratory tract.

42. The **chimeric** PIV of claim 1, wherein the vector genome or antigenome is a human-bovine **chimeric** PIV genome or antigenome.

43. The **chimeric** PIV of claim 42, wherein the human-bovine **chimeric** vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

44. The **chimeric** PIV of claim 42, wherein the vector genome or antigenome comprises a partial or complete HPIV genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) from a BPIV.

45. The **chimeric** PIV of claim 44, wherein a transcription unit comprising an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector genome or antigenome for a corresponding N ORF of a HPIV3 vector genome.

46. The **chimeric** PIV of claim 45, wherein the vector genome or antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

47. The **chimeric** PIV of claim 48, which is rHPIV3-N_B HA_{P-M}.

48. The **chimeric** PIV of claim 42, wherein the vector genome or antigenome comprises a partial or complete BPIV genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) from a HPIV.

49. The **chimeric** PIV of claim 48, wherein one or more HPIV gene(s) or genome segment(s) encoding HN and/or F **glycoproteins** or one or more immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete bovine genome or antigenome to form the vector genome or antigenome.

50. The **chimeric** PIV of claim 49, wherein both HPIV3 genes encoding HN and F **glycoproteins** are substituted for corresponding BPIV3 HN and F genes to form the vector genome or antigenome.

51. The **chimeric** PIV of claim 50, wherein the vector genome or antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a supernumerary gene insert.

52. The **chimeric** PIV of claim 51, which is selected from rBHPIV3-G1 or rB/HPIV3-F1.

53. The **chimeric** PIV of claim 49, wherein one or more HPIV1 HN and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s), fragment(s) or epitope(s) thereof are incorporated within the partial or complete bovine genome or antigenome to form the vector genome or antigenome, which is further modified by incorporation of one or more HPIV2 HN and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s), fragment(s) or epitope(s) thereof to form the **chimeric** genome or antigenome which expresses protective antigen(s) from both HPIV1 and HPIV2.

54. The **chimeric** PIV of claim 53, which is selected from rB/HPIV3.1-2F; rB/HPIV3.1-2HN; or rB/HPIV3.1-2F,2HN.

55. The **chimeric** PIV of claim 1, wherein the vector genome or antigenome is modified to encode a **chimeric glycoprotein** incorporating one or more heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV pathogen to form the **chimeric** genome or antigenome.

56. The **chimeric** PIV of claim 55, wherein the vector genome or

antigenome is modified to encode a **chimeric glycoprotein** incorporating one or more antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form the **chimeric** genome or antigenome.

57. The **chimeric** PIV of claim 55, wherein the **chimeric** genome or antigenome encodes a **chimeric glycoprotein** having antigenic domains, fragments, or epitopes from two or more HPIVs.

58. The **chimeric** PIV of claim 55, wherein the heterologous genome segment encodes a **glycoprotein** ectodomain which is substituted for a corresponding **glycoprotein** ectodomain in the vector genome or antigenome.

59. The **chimeric** PIV of claim 55, wherein one or more heterologous genome segment(s) of a second, antigenically distinct HPIV encoding said one or more antigenic domains, fragments, or epitopes is/are substituted within a HPIV vector genome or antigenome to encode said **chimeric glycoprotein**.

60. The **chimeric** PIV of claim 55, wherein heterologous genome segments encoding both a **glycoprotein** ectodomain and transmembrane region are substituted for counterpart **glycoprotein** ecto- and transmembrane domains in the vector genome or antigenome.

61. The **chimeric** PIV of claim 55, wherein said **chimeric glycoprotein** is selected from HPIV HN or F **glycoproteins**.

62. The **chimeric** PIV of claim 56, wherein the PIV vector genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct PIV is selected from HPIV1 or HPIV2.

63. The **chimeric** PIV of claim 62, wherein the HPIV vector genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct HPIV is HPIV2.

64. The **chimeric** PIV of claim 63, wherein one or more **glycoprotein** ectodomain(s) of HPIV2 is/are substituted for one or more corresponding **glycoprotein** ectodomain(s) in the HPIV3 vector genome or antigenome.

65. The **chimeric** PIV of claim 64, wherein both **glycoprotein** ectodomain(s) of HPIV2 HN and F **glycoproteins** are substituted for corresponding HN and F **glycoprotein** ectodomains in the HPIV3 vector genome or antigenome.

66. The **chimeric** PIV of claim 65, which is rPIV3-2TM.

67. The **chimeric** PIV of claim 55, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS cp45.

68. The **chimeric** PIV of claim 55, which is rPIV3-2TMcp45

69. The **chimeric** PIV of claim 55, wherein PIV2 ectodomain and transmembrane regions of one or both HN and/or F **glycoproteins** is/are fused to one or more corresponding PIV3 cytoplasmic tail region(s).

70. The **chimeric** PIV of claim 69, wherein ectodomain and transmembrane regions of both PIV2 HN and F **glycoproteins** are fused to corresponding PIV3 HN and F cytoplasmic tail regions.

71. The **chimeric** PIV of claim 70, which is rPIV3-2CT.

72. The **chimeric** PIV of claim 71, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS cp45.

73. The **chimeric** PIV of claim 72, which is rPIV3-2CTcp45.

74. The **chimeric** PIV of claim 55, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS cp45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position corresponding to Ile96 of JS cp45, a nucleotide substitution in a 3' leader sequence of the **chimeric** virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position

corresponding to nucleotide 62 of JS cp45

75. The **chimeric** PIV of claim 55, wherein a plurality of heterologous genes or genome segments encoding antigenic determinants of multiple heterologous PIVs are added to or incorporated within the partial or complete HPIV vector genome or antigenome.

76. The **chimeric** PIV of claim 75, wherein said plurality of heterologous genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and are added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

77. The **chimeric** PIV of claim 55, wherein the **chimeric** PIV genome or antigenome is attenuated by addition or incorporation of one or more gene(s) or genome segment(s) from a bovine PIV3 (BPIV3).

78. The **chimeric** PIV of claim 55, wherein the **chimeric** genome or antigenome is modified by introduction of an attenuating mutation involving an amino acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

79. The **chimeric** PIV of claim 78, wherein phenylalanine at position 456 of the HPIV3 L protein is substituted by leucine.

80. The **chimeric** PIV of claim 55, wherein the **chimeric** genome or antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinants from respiratory syncytial virus (RSV) or measles virus.

81. The **chimeric** PIV of claim 1, wherein the **chimeric** genome or antigenome is modified by addition or substitution of one or more heterologous gene(s) or genome segment(s) that confer increased genetic stability or that alter attenuation, reactogenicity in vivo, or growth in culture of the **chimeric** virus.

82. The **chimeric** PIV of claim 1, wherein the **chimeric** genome or antigenome is modified by introduction of one or more attenuating mutations identified in a biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA virus.

83. The **chimeric** PIV of claim 82, wherein the **chimeric** genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS cp45.

84. The **chimeric** PIV of claim 82, wherein the **chimeric** genome or antigenome incorporates at least one and up to a full complement of attenuating mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr_{942}, Leu_{992}, or Thr_{1558} of in JS cp45; in the N protein at a position corresponding to residues Val_{96} or Ser_{389} of JS cp45, in the C protein at a position corresponding to Ile96 of JS cp45, in the F protein at a position corresponding to residues Ile_{420} or $Ala4_{50}$ of JS cp45, in the HN protein at a position corresponding to residue Val_{384} of JS cp45, a nucleotide substitution a 3' leader sequence of the **chimeric** virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

85. The **chimeric** PIV of claim 82, wherein the **chimeric** genome or antigenome incorporates attenuating mutations from different biologically derived mutant PIVs or other mutant nonsegmented negative stranded RNA virus.

86. The **chimeric** PIV of claim 82, wherein the **chimeric** genome or antigenome incorporates an attenuating mutation at an amino acid position corresponding to an amino acid position of an attenuating mutation identified in a heterologous, mutant negative stranded RNA virus.

87. The **chimeric** PIV of claim 86, wherein said attenuating mutation comprises an amino acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

88. The **chimeric** PIV of claim 87, wherein phenylalanine at position 456 of the HPIV3 L protein is substituted by leucine.

89. The **chimeric** PIV of claim 82, wherein the **chimeric** genome or antigenome includes at least one attenuating mutation stabilized by multiple nucleotide changes in a codon specifying the mutation.

90. The **chimeric** PIV of claim 1, wherein the **chimeric** genome or antigenome comprises an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

91. The **chimeric** PIV of claim 90, wherein the additional nucleotide modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3' leader, 5' trailer, and/or intergenic region within the vector genome or antigenome or within the heterologous gene(s) or gene segment(s).

92. The **chimeric** PIV of claim 91, wherein one or more PIV gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid specified by an initiation codon, or by introduction of one or more stop codons in an open reading frame (ORF) of the gene.

93. The **chimeric** PIV of claim 92, wherein the additional nucleotide modification comprises a partial or complete deletion of one or more C, D or V ORF(s) or one or more nucleotide change(s) that reduces or ablates expression of said one or more C, D or V ORF(s).

94. The **chimeric** PIV of claim 1, wherein the **chimeric** genome or antigenome is further modified to encode a cytokine.

95. The **chimeric** PIV of claim 1, which incorporates a heterologous gene or genome segment from respiratory syncytial virus (RSV).

96. The **chimeric** PIV of claim 95, wherein the heterologous gene or genome segment encodes RSV F and/or G **glycoprotein**(s) or immunogenic domain(s), fragment(s), or epitope(s) thereof.

97. The **chimeric** PIV of claim 1 which is a virus.

98. The **chimeric** PIV of claim 1 which is a subviral particle.

99. A method for stimulating the immune system of an individual to induce protection against PIV which comprises administering to the individual an immunologically sufficient amount of the **chimeric** PIV of claim 1 combined with a physiologically acceptable carrier.

100. The method of claim 99, wherein the **chimeric** PIV is administered in a dose of 10^3 to 10^7 PFU.

101. The method of claim 99, wherein the **chimeric** PIV is administered to the upper respiratory tract.

102. The method of claim 99, wherein the **chimeric** PIV is administered by spray, droplet or aerosol.

103. The method of claim 99, wherein the vector genome or antigenome is of human PIV3 (HPIV3) and the **chimeric** PIV elicits an immune response against HPIV1 and/or HPIV2.

104. The method of claim 99, wherein the **chimeric** PIV elicits a polyspecific immune response against multiple human PIVs and/or against a human PIV and a non-PIV pathogen.

105. The method of claim 99, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

106. The method of claim 99, wherein the **chimeric** PIV elicits a polyspecific immune response against a human PIV (HPIV) and measles virus.

107. The method of claim 106, wherein the **chimeric** PIV elicits a polyspecific immune response against HPIV3 and measles virus.

108. The method of claim 99, wherein a first, **chimeric** PIV according to claim 1 and a second PIV are administered sequentially or

simultaneously to elicit a polyspecific immune response.

109. The method of claim 108, wherein the second PIV is a second, **chimeric** PIV according to claim 1.

110. The method of claim 108, wherein the first, **chimeric** PIV and second PIV are administered simultaneously in a mixture.

111. The method of claim 108, wherein the first, **chimeric** PIV and second PIV are antigenically distinct variants of HPIV.

112. The method of claim 111, wherein the first, **chimeric** PIV comprises a partial or complete HPIV3 genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a different PIV.

113. The method of claim 111, wherein the first, **chimeric** PIV and second PIV each incorporate one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a non-PIV pathogen.

114. The method of claim 113, wherein the first and second **chimeric** PIV incorporate one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of the same non-PIV pathogen.

115. A method for sequential immunization to stimulate the immune system of an individual to induce protection against multiple pathogens comprising administering to a newborn to 4 month old infant an immunologically sufficient amount of a first attenuated **chimeric** HPIV expressing an antigenic determinant of a non-PIV pathogen and one or more antigenic determinants of HPIV3 and subsequently administering an immunologically sufficient amount of a second attenuated **chimeric** HPIV expressing an antigenic determinant of a non-PIV pathogen and one or more antigenic determinants of HPIV1 or HPIV2.

116. The method for sequential immunization of claim 115, wherein the first attenuated **chimeric** HPIV is an HPIV3 expressing a measles virus antigenic determinant and wherein the second attenuated **chimeric** HPIV is a PIV3-1 **chimeric** virus expressing a measles virus antigenic determinant and incorporating one or more attenuating mutations of HPIV3 JS cp45.

117. The method for sequential immunization of claim 115, wherein following the first vaccination, the vaccinee elicits a primary antibody response against both PIV3 and the non-PIV pathogen, but not HPIV1 or HPIV2, and upon secondary immunization the vaccinee is readily infected with the second attenuated HPIV and develops both a primary antibody response to HPIV1 or HPIV2 and a high titered secondary antibody response against the non-PIV pathogen.

118. The method for sequential immunization of claim 115, wherein the first **chimeric** PIV elicits an immune response against HPIV3 and the second **chimeric** PIV elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second **chimeric** PIVs elicit an immune response against measles or RSV.

119. The method for sequential immunization of claim 115, wherein the non-PIV pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

120. The method for sequential immunization of claim 115, wherein the second **chimeric** PIV comprises a partial or complete HPIV3 vector genome or antigenome combined with one or more gene(s) or genome segment(s) encoding one or more HPIV1 and/or HPIV2 HN and/or F **glycoprotein**(s) or antigenic domain(s), fragment(s) or epitope(s) thereof.

121. The method for sequential immunization of claim 115, wherein the partial or complete vector genome or antigenome of the first, **chimeric** PIV incorporates at least one and up to a full complement of attenuating mutations present within HPIV3 JS cp45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr_{1558} of JS cp45; in the N protein at a position corresponding to residues Val96 or Ser389 of JS

cp45, in the C protein at a position corresponding to Ile96 of JS cp45, a nucleotide substitution a 3' leader sequence of the **chimeric** virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

122. An immunogenic composition to elicit an immune response against PIV comprising an immunogenically sufficient amount of the **chimeric** PIV of claim 1 in a physiologically acceptable carrier.

123. The immunogenic composition of claim 122, formulated in a dose of 10^3 to 10^7 PFU.

124. The immunogenic composition of claim 122, formulated for administration to the upper respiratory tract by spray, droplet or aerosol.

125. The immunogenic composition of claim 122, wherein the **chimeric** PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2 and HPIV3.

126. The immunogenic composition of claim 122, wherein the **chimeric** PIV elicits an immune response against HPIV3 and another virus selected from HPIV1 and HPIV2.

127. The immunogenic composition of claim 122, wherein the **chimeric** PIV elicits a polyspecific immune response against one or more HPIVs and a heterologous pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

128. The immunogenic composition of claim 127, wherein the **chimeric** PIV elicits a polyspecific immune response against HPIV3 and measles or respiratory syncytial virus

129. The immunogenic composition of claim 122, further comprising a second, **chimeric** PIV according to claim 1.

130. The immunogenic composition of claim 129, wherein the first and second **chimeric** PIVs are antigenically distinct variants of HPIV and bear the same or different heterologous antigenic determinant(s).

131. The immunogenic composition of claim 129, wherein the first **chimeric** PIV comprises a partial or complete HPIV3 genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a non-PIV heterologous pathogen.

132. The immunogenic composition of claim 129, wherein the second **chimeric** PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of the same non-PIV heterologous pathogen.

133. The immunogenic composition of claim 129, wherein the first **chimeric** PIV elicits an immune response against HPIV3 and the second **chimeric** PIV elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second **chimeric** PIVs elicit an immune response against the non-PIV pathogen.

134. The immunogenic composition of claim 129, wherein the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

135. The immunogenic composition of claim 129, wherein the heterologous pathogen is selected from measles virus or RSV.

136. The immunogenic composition of claim 129, wherein the second **chimeric** PIV comprises a partial HPIV3 vector genome or antigenome combined with one or more HPIV1 gene(s) or genome segment(s) encoding one or more antigenic determinants of HPIV1 HN and/or F **glycoproteins**.

137. The immunogenic composition of claim 129, wherein the second

chimeric PIV compresses a partial or complete HPIV3 vector genome or antigenome combined with one or more gene(s) or genome segment(s) encoding one or more HPIV2 HN and/or F **glycoprotein**(s) or antigenic domain(s), fragment(s) or epitope(s) thereof.

138. An isolated polynucleotide comprising a **chimeric** PIV genome or antigenome which includes a partial or complete PIV vector genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a **chimeric** PIV genome or antigenome.

139. The isolated polynucleotide of claim 138, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added adjacent to or within a noncoding region of the partial or complete PIV vector genome or antigenome.

140. The isolated polynucleotide of claim 138, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV vector genome or antigenome.

141. The isolated polynucleotide of claim 138, wherein said one or more heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or immunogenic fragment(s), domain(s), or epitope(s) thereof.

142. The isolated polynucleotide of claim 138, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more heterologous PIV(s).

143 The isolated polynucleotide of claim 142, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of HPIV 1 and/or HPIV2.

144. The isolated polynucleotide of claim 138, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

145. The isolated polynucleotide of claim 144, wherein said one or more heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, E, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and NS 1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

146. The isolated polynucleotide of claim 138, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome or a **chimeric** HPIV genome or antigenome comprising a partial or complete HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous HPIV added or incorporated therein.

147. The isolated polynucleotide of claim 146, wherein the heterologous pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

148. The isolated polynucleotide of claim 147, wherein a transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within a HPIV3 vector genome or antigenome.

149. The isolated polynucleotide of claim 147, wherein a transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within a HPIV3-1 vector genome or antigenome having both the HPIV3 HN and F ORFs substituted by the HN and F ORFs of HPIV1.

150. The isolated polynucleotide of claim 138, wherein the partial or complete PIV vector genome or antigenome is combined with one or more supernumerary heterologous gene(s) or genome segment(s) to form the **chimeric** PIV genome or antigenome.

151. The isolated polynucleotide of claim 150, wherein the vector genome or antigenome is a partial or complete HPIV3 genome or antigenome and said one or more supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN, HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene unit.

152. The isolated polynucleotide of claim 138, wherein one, two or all of the HPIV1 HN, HPIV2 HN, and measles virus HA ORFs are added to the vector genome or antigenome.

153. The isolated polynucleotide of claim 138, wherein one or more of the HPIV1 HN and HPIV2 HN genes and a 3918-nt GU insert is/are added are added to the vector genome or antigenome.

154. The isolated polynucleotide of claim 150, wherein said one or more supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to the wild-type HPIV3 genome length of 15,462 nt.

155. The isolated polynucleotide of claim 138, wherein the vector genome or antigenome is a human-bovine **chimeric** PIV genome or antigenome.

156. The isolated polynucleotide of claim 155, wherein the human-bovine **chimeric** vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

157. The isolated polynucleotide of claim 156, wherein the vector genome or antigenome comprises a partial or complete HPIV genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) from a BPIV.

158. The isolated polynucleotide of claim 157, wherein a transcription unit comprising an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector genome or antigenome for a corresponding N ORF of a HPIV3 vector genome.

159. The isolated polynucleotide of claim 158, wherein the vector genome or antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

160. The isolated polynucleotide of claim 138, wherein the vector genome or antigenome comprises a partial or complete BPIV genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) from a HPIV.

161. The isolated polynucleotide of claim 160, wherein one or more HPIV gene(s) or genome segment(s) encoding HN and/or F **glycoproteins** or one or more immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete bovine genome or antigenome to form the vector genome or antigenome.

162. The isolated polynucleotide of claim 161, wherein both HPIV3 genes encoding HN and F **glycoproteins** are substituted for corresponding BPIV3 HN and F genes to form the vector genome or antigenome.

163. The isolated polynucleotide of claim 162, wherein the vector genome or antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a supernumerary gene insert.

164. The isolated polynucleotide of claim 138, wherein the **chimeric** genome or antigenome encodes a **chimeric glycoprotein** having antigenic domains, fragments, or epitopes from both a human PIV (HPIV) and a heterologous pathogen.

165. The isolated polynucleotide of claim 164, wherein the **chimeric** genome or antigenome encodes a **chimeric glycoprotein** having antigenic domains, fragments, or epitopes from two or more different

PIVs.

166. The isolated polynucleotide of claim 138, wherein the **chimeric** genome or antigenome is modified by introduction of one or more attenuating mutations identified in a biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA virus.

167. The isolated polynucleotide of claim 138, wherein, the **chimeric** genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS cp45.

168. The isolated polynucleotide of claim 138, wherein the **chimeric** genome or antigenome incorporates an attenuating mutation from a heterologous nonsegmented negative stranded RNA virus.

169. The isolated polynucleotide of claim 138, wherein the **chimeric** genome or antigenome comprises an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

170. The isolated polynucleotide of claim 138, wherein the additional nucleotide modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3' leader, 5' trailer, and/or intergenic region within the vector genome or antigenome or within the heterologous gene(s) or gene segment(s).

171. The isolated polynucleotide of claim 138, wherein one or more PIV gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid specified by an initiation codon, or by introduction of one or more stop codons in an open reading frame (ORF) of the gene.

172. A method for producing an infectious attenuated **chimeric** PIV particle from one or more isolated polynucleotide molecules encoding said PIV, comprising: expressing in a cell or cell-free lysate an expression vector comprising an isolated polynucleotide comprising a partial or complete PIV vector genome or antigenome of a human or bovine PIV combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a **chimeric** PIV genome or antigenome, and PIV N, P, and L proteins.

173. The method of claim 172, wherein the **chimeric** PIV genome or antigenome and the N, P, and L proteins are expressed by two or more different expression vectors.

174. An expression vector comprising an operably linked transcriptional promoter, a polynucleotide sequence which includes a partial or complete PIV vector genome or antigenome of a human or bovine PIV combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a **chimeric** PIV genome or antigenome, and a transcriptional terminator.

175. An isolated infectious recombinant parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a PIV genome or antigenome having a polynucleotide insertion of between 150 nucleotides (nts) and 4,000 nucleotides in length in a non-coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide insertion lacking a complete open reading frame (ORF) and specifying an attenuated phenotype in said recombinant PIV.

176. The recombinant PIV of claim 175, wherein said polynucleotide insert is introduced into the PIV genome or antigenome in a reverse, non-sense orientation whereby the insert does not encode protein.

177. The recombinant PIV of claim 175, wherein said polynucleotide insert is approximately 2,000 nts or greater in length.

178. The recombinant PIV of claim 175, wherein said polynucleotide insert is approximately 3,000 nts or greater in length.

179. The recombinant PIV of claim 175, wherein said recombinant PIV replicates efficiently in vitro and exhibits an attenuated phenotype in vivo.

L15 ANSWER 18 OF 22 USPATFULL on STN
2002:279690 Virus coat protein/receptor chimeras and methods of use.
Devico, Anthony Louis, Alexandria, VA, UNITED STATES
Fouts, Timothy R., Columbia, MD, UNITED STATES
Tuskan, Robert G., Baltimore, MD, UNITED STATES
US 2002155121 A1 20021024
APPLICATION: US 2001-934060 A1 20010821 (9)
PRIORITY: US 1999-158321P 19991008 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to **chimeric** molecules comprising a virus coat sequence and a receptor sequence that can inter-act with each other to form a complex that is capable of binding a co-receptor. Such **chimeric** molecules therefore exhibit functional properties characteristic of a receptor-coat protein complex and are useful as agents that inhibit virus infection of cells due to occupancy of a co-receptor present on the cell. In particular aspects, the **chimeric** polypeptide includes an immunodeficiency virus envelope polypeptide, such as that of HIV, SIV, FIV, FeLV, FPV and herpes virus. Receptor sequences suitable for use in a **chimeric** polypeptide include, for example, CD4 D1D2 and CD4M9 sequences.

CLM What is claimed is:

1. A **chimeric** polypeptide comprising: a virus coat polypeptide sequence and a viral receptor polypeptide sequence, wherein the coat polypeptide sequence and the receptor polypeptide sequence are linked by an amino acid spacer of sufficient length to allow the coat polypeptide sequence and the viral receptor polypeptide sequence to bind to each other.

2. The **chimeric** polypeptide according to claim 1, wherein the **chimeric** polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.

3. The **chimeric** polypeptide according to claim 1, wherein the virus coat polypeptide sequence is selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 30 and SEQ ID NO: 28.

4. The **chimeric** polypeptide according to claim 3, where the receptor polypeptide sequence is selected from the group consisting of SEQ ID NO: 26 and SEQ ID NO: 20.

5. The **chimeric** polypeptide of claim 1, wherein the virus is an immunodeficiency virus selected from the group consisting of HIV. SIV, FIV, FeLV, FPV, and herpes virus.

6. The **chimeric** polypeptide of claim 1, wherein the virus coat polypeptide comprises a gp120 polypeptide sequence.

7. The **chimeric** polypeptide of claim 6, wherein the gp120 polypeptide sequence lacks 60 amino acids from the amino terminus and 20 amino acids from the carboxyl terminus.

8. The **chimeric** polypeptide of claim 1, wherein the receptor is a CD4 polypeptide sequence.

9. The **chimeric** polypeptide of claim 18, wherein the CD4 polypeptide sequence comprises the D1 and D2 domains.

10. The **chimeric** polypeptide of claim 1, wherein the spacer has from about 5 to about 200 amino acids.

11. The **chimeric** polypeptide of claim 1, wherein the spacer comprises a peptidomimetic sequence.

12. The **chimeric** polypeptide of claim 1, further comprising a heterologous domain.

13. The **chimeric** polypeptide of claim 12, wherein the heterologous domain is selected from the group consisting of: a tag, an adhesin, and an immunopotentiating agent.

14. The **chimeric** polypeptide of claim 12, wherein the heterologous domain is selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 32.

15. The **chimeric** polypeptide of claim 2, further comprising a pharmaceutically acceptable carrier.

16. The **chimeric** polypeptide of claim 4, further comprising a pharmaceutically acceptable carrier.

17. A polynucleotide sequence comprising a nucleic acid sequence encoding the **chimeric** polypeptide of claim 1.

18. The polynucleotide sequence according to claim 17, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 12.

19. The polynucleotide sequence according to claim 17, wherein a nucleic acid sequence for the virus coat polypeptide is selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 29 and SEQ ID NO: 27.

20. The polynucleotide sequence according to claim 19, wherein a nucleic acid sequence for the receptor polypeptide is selected from the group consisting of SEQ ID NO: 25 and SEQ ID NO: 19.

21. A host cell containing the expression vector of claim 18.

22. A host cell containing the expression vector of claim 20.

23. An antibody or functional fragment thereof that binds to the **chimeric** polypeptide of claim 1.

24. An antibody or functional fragment thereof that binds to the **chimeric** polypeptide of claim 2.

25. An antibody or function fragment thereof that binds to the **chimeric** polypeptide of claim 4.

26. The antibody of claim 23, wherein the antibody neutralizes the virus in vitro.

27. The antibody of claim 24, wherein the antibody neutralizes the virus in vitro.

28. The antibody of claim 25, wherein the antibody neutralizes the virus in vitro.

29. The antibody of claim 24, wherein the antibody inhibits virus infection.

30. The antibody of claim 25, wherein the antibody inhibits virus infection.

31. The antibody of claim 24, wherein the antibody binds to an epitope produced by the binding of the virus coat polypeptide sequence and the receptor polypeptide sequence.

32. The antibody of claim 25, wherein the antibody binds to an epitope produced by the binding of the virus coat polypeptide sequence and the receptor polypeptide sequence.

33. The antibody of claim 31, wherein the epitope is present on an envelope polypeptide sequence.

34. A method for producing an antibody that binds to the **chimeric** polypeptide of claim 1, comprising administering the **chimeric** polypeptide of claim 1 to a subject, or a polynucleotide that encodes the **chimeric** polypeptide of claim 1, in an amount sufficient for the subject to produce antibody to the **chimeric** polypeptide of claim 1.

35. A method for producing an antibody that binds to the **chimeric** polypeptide of claim 2, comprising administering the **chimeric** polypeptide of claim 2 to a subject, or a polynucleotide that encodes the **chimeric** polypeptide of claim 2, in an amount sufficient for the subject to produce antibody to the **chimeric** polypeptide of claim 2.

36. A method for inhibiting virus infection in a subject comprising administering to the subject an effective amount of the **chimeric** polypeptide of claim 4, or a polynucleotide encoding the **chimeric** polypeptide of claim 4, to inhibit virus infection of a cell expressing a virus co-receptor polypeptide, thereby inhibiting virus infection.

37. The method of claim 35, wherein the virus is an immunodeficiency virus.

38. The method of claim 35, wherein the subject is a human.

39. A method for producing an immune response to a virus in a subject comprising administering to the subject an effective amount of the **chimeric** polypeptide of claim 2, or a polynucleotide that encodes the **chimeric** polypeptide of claim 2, to produce an immune response to the virus.

40. The method of claim 39, wherein the virus is an immunodeficiency virus.

41. The method of claim 39, wherein the subject is a human.

42. The method of claim 39, wherein the immune response comprises an antibody.

43. The method of claim 42, wherein the antibody binds to an epitope produced by the binding of the virus coat polypeptide sequence and the receptor polypeptide sequence.

44. The method of claim 42, wherein the antibody neutralizes the virus in vitro.

45. A method for identifying an agent that inhibits an interaction between a virus and a virus co-receptor comprising the steps of: (a) contacting the **chimeric** polypeptide of claim 2 with a virus co-receptor under conditions allowing the **chimeric** polypeptide and the co-receptor to bind, in the presence and absence of a test agent; and (b) detecting binding in the presence and absence of the test agent, wherein decreased binding in the presence of the test agent thereby identifies an agent that inhibits binding between the virus and the virus co-receptor.

46. The method of claim 45, wherein the virus is an immunodeficiency virus.

47. The method of claim 45, wherein the immunodeficiency virus co-receptor is a CCR5 or CXCR4 polypeptide sequence.

48. The method of claim 45, wherein the virus co-receptor is present on the surface of an intact cell.

49. A method for identifying an agent that inhibits an interaction between a virus and a virus receptor comprising the steps of: (a) contacting the **chimeric** polypeptide of claim 2 with a test agent; and (b) detecting binding between the virus coat polypeptide sequence and the viral receptor polypeptide sequence, wherein a decreased amount of binding in the presence of the test agent identifies an agent that inhibits binding between the virus and the virus receptor.

50. The method of claim 49, wherein the test agent is selected from the group consisting of a peptide, an organic molecule, an antibody, an antiviral, an immunodeficiency virus receptor or functional fragment thereof.

51. The method of claim 50, wherein the immunodeficiency virus receptor polypeptide is a CD4 polypeptide sequence.

52. A method for identifying a **chimeric** polypeptide sequence that inhibits virus infection of a cell comprising the steps of: (a) contacting a cell susceptible to virus infection with an infectious virus particle in the presence and absence of the **chimeric** polypeptide sequence of claim 2; and (b) determining whether the **chimeric** polypeptide inhibits virus infection of the cell, thereby identifying a **chimeric** polypeptide sequence that inhibits virus infection.

53. The method of claim 52, wherein the virus is an immunodeficiency virus.

54. The method of claim 53, wherein the immunodeficiency virus is HIV.

L15 ANSWER 19 OF 22 USPATFULL on STN
2002:238646 CD4-gamma2 and CD4-IGG2 chimeras.
Maddon, Paul J., New York, NY, United States
Beaudry, Gary A., Upper Montclair, NJ, United States
Progenics Pharmaceuticals, Inc., Tarrytown, NY, United States (U.S. corporation)
US 6451313 B1 20020917

APPLICATION: US 1995-484681 19950607 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides an expression vector encoding a CD4-gamma2 **chimeric** heavy chain homodimer. This invention also provides an expression vector encoding the heavy chains of a CD4-IgG2 **chimeric** heterotetramer. Finally, this invention provides an expression vector encoding the light chains of a CD4-IgG2 **chimeric** heterotetramer.
CLM What is claimed is:
1. A purified CD4-IgG2 **chimeric** heterotetramer capable of neutralizing an HIV-1-infected individual's HIV-1 virus which comprises two heavy chains and two light chains, wherein the heavy chains are encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and have the amino acid sequence set forth in SEQ ID NO: 4; and the light chains are encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194) and have the amino acid sequence set forth in SEQ ID NO: 6.

2. A composition which comprises the purified CD4-IgG2 **chimeric** heterotetramer of claim 1 in an amount effective to neutralize an HIV-1-infected individual's HIV-1 virus and a pharmaceutically acceptable carrier.

3. A composition which consists essentially of the purified CD4-IgG2 **chimeric** heterotetramer of claim 1 in an amount effective to neutralize an HIV-1-infected individual's HIV-1 virus and a pharmaceutically acceptable carrier.

4. A composition which comprises the purified CD4-IgG2 **chimeric** heterotetramer of claim 1 linked to a toxin and a pharmaceutically acceptable carrier.

5. The composition of claim 4 wherein the toxin is selected from the group consisting of deglycosylated A chain of ricin, domain II of Pseudomonas exotoxin A, domain III of Pseudomonas exotoxin A, and Diphtheria toxin.

6. A diagnostic reagent comprising the purified CD4-IgG2 **chimeric** heterotetramer of claim 1 linked to a detectable marker.

7. The diagnostic reagent of claim 6, wherein the detectable marker is a radioisotope, chromophore or fluorophore.

8. A method of producing a purified CD4-IgG2 **chimeric** heterotetramer capable of neutralizing an HIV-1-infected individual's HIV-1 virus which comprises: a) cotransfecting a mammalian cell with an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194); b) culturing the resulting cotransfected mammalian cell under conditions and in a medium such that the **chimeric** heterotetramer is secreted into the medium; and c) recovering and purifying the CD4-IgG2 **chimeric** heterotetramer so secreted, so as to thereby produce the purified CD4-IgG2 **chimeric** heterotetramer, said heterotetramer capable of neutralizing an HIV-1-infected individual's HIV-1 virus.

9. The method of claim 8 wherein the mammalian cell is a COS cell, CHO cell or myeloma cell.

L15 ANSWER 20 OF 22 USPATFULL on STN
2002:185283 CD4-GAMMA2 AND CD4-IGG2 CHIMERAS.
BEAUDRY, GARY A., UPPER MONTCLAIR, NJ, UNITED STATES
MADDON, PAUL J., NEW YORK, NY, UNITED STATES
US 2002098191 A1 20020725
APPLICATION: US 1995-485163 A1 19950607 (8)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides an expression vector encoding a CD4-gamma2 **chimeric** heavy chain homodimer. This invention also provides an expression vector encoding the heavy chains of a CD4-IgG2 **chimeric** heterotetramer. Finally, this invention provides an expression vector encoding the light chains of a CD4-IgG2 **chimeric** heterotetramer.
CLM What is claimed is:
1. An expression vector encoding a CD4-gamma2 **chimeric** heavy chain homodimer designated CD4-IgG2-pcDNA1(ATCC No. 40952).

2. A CD4-gamma2 **chimeric** heavy chain homodimer encoded by the expression vector of claim 1.

3. A method of producing a CD4-gamma2 **chimeric** heavy chain homodimer

which comprises: a) transfecting a mammalian cell with the expression vector of claim 1; b) culturing the resulting transfected mammalian cell under conditions such that **chimeric** heavy chain homodimer is produced; and c) recovering the **chimeric** heavy chain homodimer so produced.

4. A method of claim 3, wherein the mammalian cell is a COS cell, CHO cell or myeloma cell.

5. A method of inhibiting HIV infection of a CD4+ cell which comprise treating the CD4+ cell with an amount of th CD4-gamma2 **chimeric** heavy chain homodimer of claim 2 effective to inhibit infection of the cell.

6. A method of preventing a subject from being infected with HIV which comprises administering to the subject an amount of the CD4-gamma2 **chimeric** heavy chain homodimer of claim 2 effective to prevent the subject from being infected with HIV.

7. A method of treating a subject infected with HIV so as to block the spread of HIV infection which comprises administering to the subject an amount of the CD4-gamma2 **chimeric** heavy chain homodimer of claim 2 effective to block the spread of HIV infection.

8. A pharmaceutical composition which comprises the CD4-gamma2 **chimeric** heavy chain homodimer of claim 2 in an amount effective to inhibit HIV infection of a CD4+ cell and a pharmaceutically acceptable carrier.

9. A composition of matter comprising a CD4-gamma2-**chimeric** heavy chain homodimer of claim 2 and a toxin linked thereto.

10. A composition claim 9, wherein the toxin is the deglycosylated A chain of ricin, domains II or III of Pseudomonas exotoxin A, or Diphtheria toxin.

11. A diagnostic reagent comprising a CD4-gamma2 **chimeric** heavy chain homodimer of claim 2 and a detectable marker linked thereto.

12. A diagnostic reagent of claim 11 wherein the detectable marker is a radioisotope, chromophore, or fluorophore.

13. An expression vector encoding the heavy chains of a CD4-IgG2 **chimeric** heterotetramer designated CD4-IgG2HC-pRcCMV (ATCC No. 75193).

14. An expression vector encoding the light chains of a CD4-IgG2 **chimeric** heterotetramer designated CD4-kLC-pRcCMV (ATCC No. 75194).

15. A CD4-IgG2 **chimeric** heterotetramer, the heavy chains of which are encoded by the expression vector of claim 13.

16. A CD4-IgG2 **chimeric** heterotetramer, the light chains of which are encoded by the expression vector of claim 14.

17. A CD4-IgG2 **chimeric** heterotetramer the heavy and the light chains of which are encoded by the expression vectors of claims 13 and 14, respectively.

18. A method of producing a CD4-IgG2 **chimeric** heterotetramer which comprises: a) cotransfecting a mammalian cell with the expression vector of claim 13 and an expression vector encoding a light chain; b) culturing the resulting cotransfected mammalian cell under conditions such that the CD4-IgG2 **chimeric** heterotetramer is produced; and c) recovering the CD4-IgG2 **chimeric** heterotetramer so produced.

19. A method of producing an CD4-IgG2 **chimeric** heterotetramer which comprises: a) cotransfecting a mammalian cell with the expression vector of claim 14 and an expression vector encoding an IgG2 heavy chain and; b) culturing the resulting cotransfected mammalian cell under conditions such that the **chimeric** heterotetramer is produced; and c) recovering the **chimeric** heterotetramer so produced.

20. A method of producing a CD4-IgG2 **chimeric** heterotetramer which comprises: a) cotransfecting a mammalian cell with the expression vectors of claim 13 and 14; b) culturing the resulting cotransfected mammalian cell under conditions such that the **chimeric** heterotetramer is produced; and c) recovering the **chimeric** heterotetramer so produced.

21. A method of claim 18, 19 or 20, wherein the mammalian cell is a COS cell, CHO or myeloma cell.

22. A method of inhibiting HIV infection of a CD4+ cell which comprises treating the CD4+ cell with an amount of the CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 effective to inhibit infection of the cell.

23. A method of preventing a subject from being infected with HIV which comprises administering to the subject an amount of the CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 effective to prevent the subject from being infected with HIV.

24. A method of treating a subject infected with HIV so as to block the spread of HIV infection which comprises administering to the subject an amount of CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 effective to block spread of HIV infection.

25. A pharmaceutical composition which comprises the CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 in an amount effective to inhibit HIV infection of a CD4+ cell and a pharmaceutically acceptable carrier.

26. A composition of matter comprising a CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 and a toxin linked thereto.

27. A composition of claim 26, wherein the toxin is the deglycosylated A chain of ricin, domains II or III of Pseudomonas exotoxin A, and Diphtheria toxin.

28. A diagnostic reagent comprising a CD4-IgG2 **chimeric** heterotetramer of claim 15, 16 or 17 and a detectable marker linked thereto.

29. A diagnostic reagent of claim 28 wherein the detectable marker is a radioisotope, chromophore or fluorophore.

L15 ANSWER 21 OF 22 USPATFULL on STN
2002:141109 Death domain containing receptor 5.
Ni, Jian, Rockville, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Rosen, Craig A., Laytonville, MD, UNITED STATES
Human Genome Sciences, Inc., Rockville, MD, 20850 (U.S. corporation)
US 2002072091 A1 20020613
APPLICATION: US 2001-874138 A1 20010606 (9)
PRIORITY: US 1999-148939P 19990813 (60)
US 1999-133238P 19990507 (60)
US 1999-132498P 19990504 (60)
US 1997-40846P 19970317 (60)
US 1997-54021P 19970729 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel Death Domain Containing Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid molecules are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying antagonists and antagonists of DR5 activity.

CLM What is claimed is:
1. A method for treating graft versus host disease, viral infection, cancer, leukemia, immunodeficiency, or an autoimmune disorder comprising administering to an individual therapeutically effective amounts of: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO: 2; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

2. The method of claim 1, wherein said first therapeutic agent comprises an antibody which binds to a polypeptide consisting of amino acids I to 133 of SEQ ID NO: 2.

3. The method of claim 1, wherein said antibody is a monoclonal antibody.

4. The method of claim 1, wherein said antibody is a polyclonal antibody.

5. The method of claim 1, wherein said antibody is a **chimeric** antibody.

6. The method of claim 1, wherein said antibody is a humanized antibody.

7. The method of claim 1, wherein said antibody is a single-chain Fv antibody.

8. The method of claim 1, wherein said antibody is an Fab antibody fragment.

9. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at the same time.

10. The method of claim 1, wherein said first and second therapeutic agents are administered to the individual at different times.

11. The method of claim 1, wherein said second therapeutic agent is TRAIL.

12. The method of claim 1, wherein said tumor necrosis factor blocking agent comprises an antibody which binds to a protein selected from the group consisting of: (a) TNF-α; (b) TNF-β; (c) TNF-γ; (d) TNF-γ-α; and (e) TNF-γ-β.

13. The method of claim 1, wherein said immunosuppressive agent is selected from the group consisting of: (a) cyclosporine; (b) cyclophosphamide; (c) methylprednisone; (d) prednisone; (e) azathioprine; (f) FK-506; and (g) 15-deoxyspergualin.

14. The method of claim 1, wherein said cytokine is selected from the group consisting of: (a) IL-2; (b) IL-3; (c) IL-4; (d) IL-5; (e) IL-6; (f) IL-7; (g) IL-10; (h) IL-12; (i) IL-13; (j) IL-15; and (k) IFN-γ.

15. A composition comprising: (a) a first therapeutic agent comprising an antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO: 2; and (b) a second therapeutic agent selected from the group consisting of: (i) TRAIL; (ii) a tumor necrosis factor; (iii) a tumor necrosis factor blocking agent; (iv) an immunosuppressive agent; (v) an antibiotic; (vi) an anti-inflammatory agent; (vii) a chemotherapeutic agent; and (viii) a cytokine.

16. The composition of claim 15, which further comprises a pharmaceutically acceptable carrier or excipient.

17. An isolated polypeptide comprising an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO.2; wherein said polypeptide is covalently attached to polyethylene glycol, said polyethylene glycol having an average molecule weight selected from the group consisting of 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, and 20,000

18. The polypeptide of claim 17, comprising an amino acid sequence at least 95% identical to amino acids 1 to 133 of SEQ ID NO-2.

19. The polypeptide of claim 18, wherein said amino acid sequence comprises amino acids 1 to 133 of SEQ ID NO: 2.

20. The polypeptide of claim 17, wherein said polypeptide has an average degree of substitution with polyethylene glycol which falls within a range selected from the group consisting of 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, and 10-12.

21. The polypeptide of claim 17, which is produced by a recombinant host cell.

22. The polypeptide of claim 21, wherein said recombinant host cell which is a eukaryotic host cell.

23. The polypeptide of claim 17, which comprises a heterologous polypeptide.

24. The polypeptide of claim 23, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

25. A composition comprising the polypeptide of claim 17 and a pharmaceutically acceptable carrier.

26. An isolated polynucleotide comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO: 2.

27. The polynucleotide of claim 26, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to amino acids 1 to 133 of SEQ ID NO: 2.

28. The polynucleotide of claim 27, comprising a nucleic acid encoding amino acids 1 to 133 of SEQ ID NO: 2.

29. The polynucleotide of claim 26, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids 1 to 360 of SEQ ID NO: 2.

30. The polynucleotide of claim 29, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to amino acids 1 to 360 of SEQ ID NO: 2.

31. The polynucleotide of claim 30, comprising a nucleic acid encoding amino acids 24 to 468 of SEQ ID NO: 2.

32. The polynucleotide of claim 29, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to amino acids -51 to 360 of SEQ ID NO: 2.

33. The polynucleotide of claim 32, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to amino acids -51 to 360 of SEQ ID NO: 2.

34. The polynucleotide of claim 33, comprising a nucleic acid encoding amino acids -51 to 360 of SEQ ID NO: 2.

35. The polynucleotide of claim 26, further comprising a heterologous polynucleotide.

36. The polynucleotide of claim 35, wherein said heterologous polynucleotide encodes a heterologous polypeptide.

37. The polynucleotide of claim 26, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

38. A method of producing a vector which comprises inserting the polynucleotide of claim 26 into a vector.

39. A vector comprising the polynucleotide of claim 26.

40. The vector of claim 39, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

41. A host cell comprising the polynucleotide of claim 26.

42. The host cell of claim 41, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

43. A method of producing a polypeptide which comprises culturing the host cell of claim 32 under conditions such that said polypeptide is expressed, and recovering said polypeptide.

44. An isolated polynucleotide comprising a nucleic acid encoding an amino acid sequence at least 90% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

45. The polynucleotide of claim 44, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

46. The polynucleotide of claim 45, comprising a nucleic acid encoding the mature polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

47. The polynucleotide of claim 44, comprising a nucleic acid encoding an amino acid sequence at least 90% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

48. The polynucleotide of claim 47, comprising a nucleic acid encoding an amino acid sequence at least 95% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

49. The polynucleotide of claim 48, comprising a nucleic acid a nucleic acid encoding the complete polypeptide encoded by the cDNA clone in ATCC Deposit No. 97920.

50. The polynucleotide of claim 44, further comprising a heterologous polynucleotide.

51. The polynucleotide of claim 50, wherein said heterologous polynucleotide encodes a heterologous polypeptide.

52. The polynucleotide of claim 51, wherein said heterologous polynucleotide encodes an Fc portion of an antibody.

53. A method of producing a vector which comprises inserting the polynucleotide of claim 44 into a vector.

54. A vector comprising the polynucleotide of claim 44.

55. The vector of claim 54, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

56. A host cell comprising the polynucleotide of claim 44.

57. The host cell of claim 56, wherein said polynucleotide is operably associated with a heterologous regulatory polynucleotide.

58. A method of producing a polypeptide which comprises culturing the host cell of claim 57 under conditions such that said polypeptide is expressed, and recovering said polypeptide.

59. An isolated polypeptide comprising an amino acid sequence at least 90% identical to amino acids 1 to 133 of SEQ ID NO: 2.

60. The polypeptide of claim 59, wherein said amino acid sequence is at least 95% identical to amino acids 1 to 133 of SEQ ID NO: 2.

61. The polypeptide of claim 60, wherein said amino acid sequence comprises amino acids 1 to 133 of SEQ ID NO.2.

62. The polypeptide of claim 59, wherein said amino acid sequence is at least 90% identical to amino acids 1 to 133 of SEQ ID NO: 2.

63, The polypeptide of claim 62, wherein said amino acid sequence is at least 95% identical to amino acids 1 to 360 of SEQ ID NO: 2.

64. The polypeptide of claim 63, wherein said amino acid sequence comprises amino acids 1 to 360 of SEQ ID NO: 2.

65. The polypeptide of claim 62, wherein said amino acid sequence is at least 90% identical to amino acids -51 to 360 of SEQ ID NO: 2.

66. The polypeptide of claim 65, wherein said amino acid sequence is at least 95% identical to amino acids -51 to 360 of SEQ ID NO: 2.

67. The polypeptide of claim 66, wherein said amino acid sequence comprises amino acids -51 to 360 of SEQ ID NO: 2.

68. The polypeptide of claim 59, which is produced by a recombinant host cell.

69. The polypeptide of claim 68, wherein said recombinant host cell which is a eukaryotic host cell.

70. The polypeptide of claim 59, which comprises a heterologous polypeptide.

71. The polypeptide of claim 70, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

72. A composition comprising the polypeptide of claim 59 and a pharmaceutically acceptable carrier.

73. An isolated polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence of the mature polypeptide

encoded by the cDNA clone contained in ATCC Deposit No. 97920.

74. The polypeptide of claim 73, which comprises an amino acid sequence at least 95% identical to the amino acid sequence of the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No 97920.

75. The polypeptide of claim 74, which comprises the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No.97920.

76. The polypeptide of claim 73, which comprises an amino acid sequence at least 90% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No.97920.

77. The polypeptide of claim 76, which comprises an amino acid sequence at least 95% identical to the amino acid sequence of the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No.97920.

78. The polypeptide of claim 77, which comprises the complete polypeptide encoded by the cDNA clone contained in ATCC Deposit No.97920.

79. The polypeptide of claim 73, which is produced by a recombinant host cell.

80. The polypeptide of claim 79, wherein said recombinant host cell which is a eukaryotic host cell.

81. The polypeptide of claim 73, which comprises a heterologous polypeptide.

82. The polypeptide of claim 81, wherein said heterologous polypeptide comprises an Fc portion of an antibody.

83. A composition comprising the polypeptide of claim 73 and a pharmaceutically acceptable carrier.

84. An isolated antibody which binds to a polypeptide consisting of amino acids -51 to 360 of SEQ ID NO: 2.

85. The antibody of claim 84, wherein said antibody is a monoclonal antibody.

86. The antibody of claim 84, wherein said antibody is a polyclonal antibody.

87. The antibody of claim 84, wherein said antibody is an Fab antibody fragment.

88. The antibody of claim 84, wherein said antibody is an F(ab')2 antibody fragment.

89. A method for treating a disease or condition selected from the group consisting of. (a) cancer; (b) inflammation; (c) an autoimmune disease; and (d) graft v. host disease, wherein said method comprises administering to an individual a therapeutically effective amount of the antibody of claim 84.

90. A composition comprising the antibody of claim 84 and a pharmaceutically acceptable carrier.

91. An isolated antibody which binds to a polypeptide consisting of the amino acid sequence of the complete polypeptide encoded by the DNA clone contained in ATCC Deposit No. 97920.

92. The antibody of claim 91, wherein said antibody is a monoclonal antibody.

93. The antibody of claim 91, wherein said antibody is a polyclonal antibody.

94. The antibody of claim 91, wherein said antibody is an Fab antibody fragment.

95. The antibody of claim 91, wherein said antibody is an F(ab')2 antibody fragment.

96. A method for treating a disease or condition selected from the group consisting of. (a) cancer; (b) inflammation; (c) an autoimmune disease; and (d) graft versus host disease, wherein said method comprises administering to an individual a therapeutically effective amount of the antibody of claim 91.

97. A composition comprising the antibody of claim 91 and a pharmaceutically acceptable carrier.

L15 ANSWER 22 OF 22 USPATFULL on STN
2001:22194 Uses of CD4-gamma2 and CD4-IgG2 chimeras.
Maddon, Paul J., New York, NY, United States
Beaudry, Gary A., Upper Montclair, NJ, United States
Progenics Pharmaceuticals, Inc., Tarrytown, NY, United States (U.S. corporation)
US 6187748 B1 20010213
APPLICATION: US 1995-485372 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides the CD4-IgG2 **chimeric** heterotetramer, wherein the heavy chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193). This invention also provides the CD4-IgG2 **chimeric** heterotetramer, wherein the light chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194). This invention also provides the CD4-IgG2 **chimeric** heterotetramer, wherein the heavy chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and the light chains of the **chimeric** heterotetramer is encoded by the expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194). Finally, this invention provides a method of inhibiting HIV infection of a CD4+ cell, a method of preventing a subject from being infected with HIV, and a method of treating a subject infected with HIV so as to block the spread of HIV infection, using the above CD-4-IgG2 **chimeric** heterotetramers.
CLM What is claimed is:
1. A method of inhibiting infection of a CD4+ cell by a human immunodeficiency virus which comprises contacting the human immunodeficiency virus with an amount of a CD4-IgG2 **chimeric** heterotetramer effective to form a complex with all such human immunodeficiency virus which is in the presence of the CD4+ cell so as to thereby inhibit infection of the CD4+ cell by the virus, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, the heavy chains being encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and the light chains being encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

2. A method of preventing CD4+ cells of a subject from becoming infected with human immunodeficiency virus which comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to bind to any human immunodeficiency virus present in the subject, so as to thereby prevent the subject's CD4+ cells from becoming infected with human immunodeficiency virus, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, the heavy chains being encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and the light chains being encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

3. A method of treating a subject having CD4+ cells infected with human immunodeficiency virus which comprises administering to the subject an amount of a CD4-IgG2 **chimeric** heterotetramer effective to bind to any human immunodeficiency virus present in the subject, so as to thereby treat the subject having CD4+ cells infected with human immunodeficiency virus, wherein the CD4-IgG2 **chimeric** heterotetramer comprises two heavy chains and two light chains, the heavy chains being encoded by an expression vector designated CD4-IgG2HC-pRcCMV (ATCC No. 75193) and the light chains being encoded by an expression vector designated CD4-kLC-pRcCMV (ATCC No. 75194).

=> d his

(FILE 'HOME' ENTERED AT 18:22:37 ON 24 JUL 2005)

FILE 'USPATFULL' ENTERED AT 18:23:32 ON 24 JUL 2005
L1 2139 S (FILOVIR? OR MARBURG OR EBOLA)

```
L2           35 S L1 AND (GP1 OR GP2)
L3          255 S L1 AND (BIVALENT OR MULTIVALENT)
L4            8 S L3 AND (MARBURG/TI OR EBOLA/TI OR FILOVIR?/TI)
L5            6 S L4 NOT L2
                E GROGAN CASE C/IN
L6            1 S E3
                E SCHMALJOHN ALAN L/IN
L7           11 S E2 OR E3
L8           10 S L7 NOT L6

     FILE 'WPIDS' ENTERED AT 18:38:30 ON 24 JUL 2005
L9          329 S (FILOVIR? OR MARBURG OR EBOLA)
L10           5 S L9 AND (MULTIVALENT OR BIVALENT)

     FILE 'MEDLINE' ENTERED AT 18:39:46 ON 24 JUL 2005

     FILE 'USPATFULL' ENTERED AT 18:39:53 ON 24 JUL 2005
L11      1167 S DENGUE VIRUS?
L12       696 S L11 AND (GP OR GLYCOPROTEIN?)
L13       149 S L12 AND (BIVALENT OR MULTIVALENT)
L14       106 S L13 AND CHIMERIC
L15        22 S L14 AND CHIMERIC/CLM

=> s l13 and (dengue/clm or DEN/clm)
           229 DENGUE/CLM
           134 DEN/CLM
L16        32 L13 AND (DENGUE/CLM OR DEN/CLM)

=> s l16 not l15
L17        30 L16 NOT L15

=> d l17,cbib,ab,clm,1-130

L17  ANSWER 1 OF 30  USPATFULL on STN
2005:4300 Polyvalent protein complex.
    Rossi, Edmund A., Nutley, NJ, UNITED STATES
    Chang, Chien Hsing, Downingtown, PA, UNITED STATES
    McBride, William John, Boonton, NJ, UNITED STATES
    US 2005003403 A1 20050106
    APPLICATION: US 2004-829388  A1  20040422 (10)
    PRIORITY: US 2003-464532P  20030422 (60)
    US 2003-525391P  20031124 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention provides for a polyvalent protein complex (PPC) comprising
       two polypeptide chains generally arranged laterally to one another. Each
       polypeptide chain typically comprises 3 or 4 "v-regions", which comprise
       amino acid sequences capable of forming an antigen binding site when
       matched with a corresponding v-region on the opposite polypeptide chain.
       Up to about 6 "v-regions" can be used on each polypeptide chain. The
       v-regions of each polypeptide chain are connected linearly to one
       another and may be connected by interspersed linking regions. When
       arranged in the form of the PPC, the v-regions on each polypeptide chain
       form individual antigen binding sites.
CLM    What is claimed is:
       1. A polyvalent protein complex (PPC) comprising a first and a second
       polypeptide chain,  wherein said first polypeptide chain comprises a
       polypeptide sequence represented, by the formula a1-11-a2-
       l2-a3,  wherein a1, a2, and a3 are
       immunoglobulin variable domains and l1 and l2 are peptide
       linkers, and a1 is N-terminal of a2, which in turn is
       N-terminal of a3,  wherein said second polypeptide chain comprises
       a polypeptide sequence represented by the formula b1-13-
       b2-14-b3,  wherein b1, b2, and b3 are
       immunoglobulin variable domains and l3 and l4 are peptide
       linkers, and b3 is N-terminal of b2, which in turn is
       N-terminal of b1,  wherein said first and second polypeptide chain
       together form a complex comprising at least three antigen binding sites,
       wherein each of said antigen binding sites comprises a variable domain
       from said first polypeptide chain and a variable domain from said second
       polypeptide chain, and  wherein each binding site comprises an
       immunoglobulin heavy chain variable domain and an immunoglobulin light
       chain variable domain.

       2. The complex according to claim 1 wherein each polypeptide chain
       further comprises 1-3 additional immunoglobulin variable domains,
       wherein each domain is linked via a peptide linker,  wherein said first
       and second polypeptide chain together form a complex comprising 4-6
```

antigen binding sites, and wherein each of said antigen binding sites comprises a variable domain from said first polypeptide chain and a variable domain from said second polypeptide chain.

3. The complex according to claim 1, wherein at least one polypeptide chain further comprises an amino acid sequence selected from the group consisting of a toxin, a cytokine, a lymphokine, a enzyme, a growth factor, and an affinity purification tag.

4. The complex according to claim 1, wherein at least two of said antigen binding sites have the same binding specificity.

5. The complex according to claim 1, wherein each of said antigen binding sites has a different binding specificity.

6. The complex according to claim 4, wherein said antigen binding sites have the same binding specificity.

7. The complex according to claim 2 wherein said antigen binding sites have at least two different binding specificities.

8. The complex according to claim 7 wherein at least 3 of said antigen binding sites have different binding specificities.

9. The polyvalent protein complex of claim 7 wherein at least 4 of said antigen binding sites have different binding specificities.

10. The complex according to claim 7 comprising at least 5 antigen binding sites wherein at least 5 of said binding sites have different binding specificities.

11. The complex according to claim 7 comprising 6 antigen binding sites each having a different binding specificity.

12. The complex according to claim 7 comprising at least 5 antigen binding sites wherein at least 5 of said binding sites have different binding specificities.

13. The complex according to claim 1, wherein two of said antigen binding sites are specific for epitopes of tumor associated antigens, and wherein said third antigen binding sites is reactive with a targetable construct.

14. The polyvalent protein complex of claim 13, wherein two antigen binding sites are specific for epitopes of tumor associated antigens, and wherein the third antigen binding sites is reactive with a targetable construct, and wherein the epitope on the targetable construct is a hapten.

15. A complex comprising at least one complex according to claim 1 bound to a targetable construct, wherein said complex is bound to a first hapten on said construct and wherein said construct further comprises a second hapten capable of binding simultaneously to a second polyvalent protein complex.

16. The polyvalent protein complex of claim 14, wherein the tumor associated antigen, or antigens are selected from the group consisting of antigens associated with carcinomas, melanomas, sarcomas, gliomas, leukemias and lymphomas.

17. The polyvalent protein complex of claim 14, wherein the tumor associated antigen is selected from the group consisting of α-fetoprotein, A3, CA125, carcinoembryonic antigen (CEA), CD19, CD20, CD21, CD22, CD23, CD30, CD33, CD45, CD74, CD80, colon-specific antigen-p (CSAp), EGFR, EGP-1, EGP-2, folate receptor, HER2/neu, HLA-DR, human chorionic gonadrotropin, Ia, IL-2, IL-6, insulin-like growth factor, KS-1, Le(y), MAGE, MUC1, MUC2, MUC3, MUC4, NCA66, necrosis antigens, PAM-4, placental growth factor, prostatic acid phosphatase PSA, PSMA, S100, T101, TAC, TAG-72, tenascin and VEGF.

18. The polyvalent protein complex of claim 16, comprising at least two tumor antigen binding sites, wherein both tumor antigen binding sites are specific for CEA and wherein the third binding site is specific for the hapten, histamine-succinyl-glycine (HSG).

19. The polyvalent protein complex of claim 16, wherein the polyvalent protein is BS14HP, or hBS14.

20. A complex comprising a polyvalent protein complex according to claim

19, bound to IMP 241, or IMP 245

21. A pretargeting method of treating or diagnosing or treating and diagnosing a neoplastic condition comprising (a) administering to said subject the polyvalent protein complex of claim 1, wherein two antigen binding sites are directed to a tumor associated antigen, and one antigen binding sites is directed to a targetable construct comprising a **bivalent** hapten; (b) optionally, administering to said subject a clearing composition, and allowing said composition to clear the polyvalent complex from circulation; and (c) administering to said subject said targetable construct comprising a **bivalent** hapten, wherein said targetable construct further comprises one or more chelated or chemically bound therapeutic or diagnostic agents.

22. The method of claim 21, wherein the diagnostic agent is a radionuclide selected from the group consisting of ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154-158}Gd$, ^{177}Lu, ^{32}P, ^{188}Re, and ^{90}Y or a combination thereof.

23. The method of claim 22, wherein said radioactive labels are imaged using computed tomography (CT), single photon emission computed tomography (SPECT), or positron emission tomography (PET).

24. The method of claim 22, wherein the application is for intraoperative diagnosis to identify occult neoplastic tumors.

25. The method of claim 21, wherein said targetable construct comprises one or more image enhancing agents for use in magnetic resonance imaging (MRI).

26. The method of claim 25, wherein said image enhancing agent is a metal selected from the group consisting of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (III), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III).

27. The method of claim 21, wherein said targetable construct comprises one or more image enhancing agents for use in ultrasound imaging.

28. The method of claim 21, wherein said targetable construct is a liposome with a **bivalent** HSG-peptide covalently attached to the outside surface of the liposome lipid membrane.

29. The method of claim 28, wherein said liposome is gas filled.

30. The method of claim 21, wherein said targetable construct comprises one or more radioactive isotopes useful for killing neoplastic cells.

31. The method of claim 30, wherein said radioactive isotope is selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac or a combination thereof.

32. The method of claim 30, wherein the pretargeted therapy is administered prior to, with or after one or more therapeutic agents.

33. The method of claim 32, wherein said therapeutic agent is a cytokine or a chemotherapeutic agent, or a colony-stimulating growth factor.

34. The method of claim 33, wherein said therapeutic agent is a chemotherapeutic agent selected from the group consisting of taxanes, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes; folic acid analogs, pyrimidine analogs, purine analogs, vinca alkaloids, antibiotics, enzymes, platinum coordination complexes, substituted urea, methyl hydrazine derivatives, adrenocortical suppressants, and antagonists.

35. The method of claim 33, wherein said therapeutic agent is a chemotherapeutic agent selected from the group consisting of steroids, progestins, estrogens, antiestrogens, and androgens.

36. The method of claim 33, wherein said therapeutic agent is a chemotherapeutic agent selected from the group consisting of azaribine,

bleomycin, bryostatin-1, busulfan, carmustine, chlorambucil, cisplatin, CPT-11, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, ethinyl estradiol, etoposide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, methotrexate, methotrexate, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, uracil mustard, vinblastine, and vincristine.

37. The method of claim 33, wherein said therapeutic agent is a cytokine selected from the group consisting of interleukin-1 (IL-1), IL-2, IL-3, IL-6, IL-10, IL-12, interferon-alpha, interferon-beta, and interferon-gamma.

38. The method of claim 33, wherein said therapeutic agent is a colony-stimulating growth factor selected from the group consisting of granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), erthropoietin and thrombopoietin.

39. A method of treating a neoplastic disorder in a subject, comprising administering to said subject a "naked" polyvalent protein complex according to claim 1, wherein at least one of said antigen binding sites binds to an antigen selected from the group consisting of alpha fetoprotein, A3, CA125, carcinoembryonic antigen (CEA), CD19, CD20, CD21, CD22, CD23, CD30, CD33, CD45, CD74, CD80, colon-specific antigen-p (CSAp), EGFR, EGP-1, EGP-2, folate receptor, HER2/neu, HLA-DR, human chorionic gonadrotropin, Ia, IL-2, IL-6, insulin-like growth factor, KS-1, Le(y), MAGE, MUC1, MUC2, MUC3, MUC4, NCA66, necrosis antigens, PAM-4, placental growth factor, prostatic acid phosphatase PSA, PSMA, S100, T101, TAC, TAG-72, tenascin and VEGF.

40. The method of claim 39, wherein the neoplastic disorder is selected from the group consisting of carcinomas, sarcomas, gliomas, lymphomas, leukemias, and melanomas.

41. A method for treating a B-cell malignancy, or B-cell immune or autoimmune disorder in a subject, comprising administering to said subject one or more dosages of a therapeutic composition comprising a polyvalent protein complex of claim 1 and a pharmaceutically acceptable carrier.

42. A method for treating a B-cell malignancy, or B-cell immune or autoimmune disorder in a subject, comprising administering to said subject one or more dosages of a therapeutic composition comprising a polyvalent protein complex of claim 2 and a pharmaceutically acceptable carrier, wherein each antigen binding site binds a distinct epitope of CD19, CD20 or CD22.

43. The method of claim 42, wherein said polyvalent protein complex is parenterally administered in a dosage of 20 to 1500 milligrams protein per dose.

44. The method of claim 42, wherein said polyvalent protein complex is parenterally administered in a dosage of 20 to 500 milligrams protein per dose.

45. The method of claim 42, wherein said polyvalent protein complex is parenterally administered in a dosage of 20 to 100 milligrams protein per dose.

46. The method of claim 42, wherein said subject receives the polyvalent protein complex as repeated parenteral dosages of 20 to 100 milligrams protein per dose.

47. The method of claim 42, wherein said subject receives the polyvalent protein complex as repeated parenteral dosages of 20 to 1500 milligrams protein per dose.

48. The method of claim 42, wherein a sub-fraction of the polyvalent protein complex is labeled with a radioactive isotope.

49. The method of claim 48, wherein said radioactive isotope is selected from the group consisting of ^{32}P, ^{33}P, ^{47}Sc, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{90}Y, ^{111}Ag, ^{111}In, ^{125}I, ^{131}I, ^{142}Pr, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho,

^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac or a combination thereof.

50. A method for detecting or diagnosing a B-cell malignancy, or B-cell immune or autoimmune disorder in a subject, comprising administering to said subject a diagnostic composition comprising a polyvalent protein complex of claim 2 and a pharmaceutically acceptable carrier, wherein each antigen binding site binds a distinct epitope of CD19, CD20 or CD22, and wherein said complex is radiolabeled with a radionuclide selected from the group consisting of ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154-158}Gd$, ^{177}Lu, ^{32}P, 188Re, and ^{90}Y or a combination thereof.

51. The method of claim 50, wherein said radioactive labels are imaged using computed tomography (CT), single photon emission computed tomography (SPECT), or positron emission tomography (PET).

52. The method of claim 50, wherein the application is for intraoperative diagnosis to identify occult neoplastic tumors.

53. A method for detecting or diagnosing a B-cell malignancy, or B-cell immune or autoimmune disorder in a subject, comprising administering to said subject a diagnostic composition comprising a polyvalent protein complex of claim 2 and a pharmaceutically acceptable carrier, wherein each antigen binding site binds a distinct epitope of CD19, CD20 or CD22, and wherein said complex is labeled with one or more image enhancing agents for use in magnetic resonance imaging (MRI).

54. The method of claim 53, wherein said image enhancing agent is a paramagnetic ion selected from the group consisting of chromium (III), manganese (II), iron (II), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III).

55. A method of diagnosing a non-neoplastic disease or disorder, comprising administering to a subject suffering from said disease or disorder a complex according to claim 1, wherein a detectable label is attached to said complex, and wherein one or more of said antigen binding sites is specific for a marker substance of the disease or disorder.

56. The method of claim 55, wherein said disease or disorder is caused by a fungus.

57. The method of claim 56, wherein said fungus is selected from the group consisting of Microsporum, Trichophyton, Epidermophyton, Sporothrix schenckii, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis, and Candida albicans.

58. The method of claim 55 wherein said disease or disorder is caused by a virus.

59. The method of claim 58, wherein said virus is selected from the group consisting of human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, **Dengue virus**, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus and blue tongue virus.

60. The method of claim 55 wherein said disease or disorder is caused by a bacterium.

61. The method of claim 60, wherein said bacterium is selected from the group consisting of Anthrax bacillus, Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, and Mycobacterium tuberculosis

62. The method of claim 55 wherein said disease or disorder is caused by

a Mycoplasma.

63. The method of claim 55 wherein said disease or disorder is caused by a parasite.

64. The method of claim 55 wherein said disease or disorder is malaria.

65. The method of claim 55, wherein said disease or disorder is an autoimmune disease.

66. The method of claim 65, wherein said autoimmune disease is selected from the group consisting of acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcalnephritis, erythema nodosurn, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, multiple sclerosis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitisubiterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis, psoriasis, and fibrosing alveolitis.

67. The method of claim 55, wherein said the disease or disorder is myocardial infarction, ischemic heart disease, or atherosclerotic plaques.

68. The method of claim 55, wherein said disease or disorder is graft rejection.

69. The method of claim 55, wherein said disease or disorder is Alzheimer's disease.

70. The method of claim 55, wherein said disease or disorder is caused by atopic tissue.

71. The method of claim 55, wherein said disease or disorder is inflammation caused by accretion of activated granulocytes, monocytes, lymphoid cells or macrophages at the site of inflammation, and wherein the inflammation is caused by an infectious agent.

72. The method of claim 55, wherein said detectable label is a radionuclide selected from the group consisting of ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154-158}Gd$, ^{177}Lu, ^{32}P, ^{188}Re, and ^{90}Y or a combination thereof.

73. The method of claim 72, wherein said radioactive labels are imaged using computed tomography (CT), single photon emission computed tomography (SPECT), or positron emission tomography (PET).

74. The method of claim 73, wherein the application is for intraoperative diagnosis of said disease or disorder.

75. The method of claim 55, wherein at least one of said antigen binding sites is specific for a targetable construct, and wherein said construct comprises one or more image enhancing agents for use in magnetic resonance imaging (MRI).

76. The method of claim 75, wherein said image enhancing agent is a paramagnetic ion selected from the group consisting of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III).

77. The method of claim 55, wherein at least one of said antigen binding sites is specific for a targetable construct, and wherein said targetable construct comprises one or more image enhancing agents for use in ultrasound imaging.

78. The method of claim 55, wherein at least one of said antigen binding sites is specific for a targetable construct and wherein said targetable

construct comprises a liposome with a **bivalent** HSG-peptide covalently attached to the outside surface of the liposome lipid membrane.

79. The method of claim 74, wherein said liposome is gas filled.

80. A pretargeting method of treating or diagnosing a non-neoplastic disease or disorder in a subject comprising (a) administering to said subject the polyvalent protein complex of claim 1, wherein two antigen binding sites are directed to a marker substance, or marker substances specific for the disorder, and one antigen binding sites is directed to a targetable construct comprising a **bivalent** hapten; (b) optionally administering to said subject a clearing composition, and allowing said composition to clear the polyvalent complex from circulation; and (c) administering to said subject said targetable construct comprising a **bivalent** hapten, wherein the targetable construct further comprises one or more chelated or chemically bound therapeutic or diagnostic agents.

81. The method of claim 80, wherein said disease or disorder is caused by a fungus.

82. The method of claim 81, wherein the species of fungus is selected from the group consisting of Microsporum, Trichophyton, Epidermophyton, Sporothrix schenckii, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis, or Candida albicans.

83. The method of claim 80 wherein said disease or disorder is caused by a virus.

84. The method of claim 83, wherein the species of virus is selected from the group consisting of human immunodeficiency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, **Dengue virus**, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningifis virus, wart virus and blue tongue virus.

85. The method of claim 80 wherein said disease or disorder is caused by a bacterium.

86. The method of claim 85, wherein the bacterium is selected from the group consisting of Anthrax bacillus, Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, and Mycobacterium tuberculosis.

87. The method of claim 80 wherein said disease or disorder is caused by a Mycoplasma.

88. The method of claim 80 wherein said disease or disorder is caused by a parasite.

89. The method of claim 80 wherein the disease or disorder is malaria.

90. The method of claim 80, wherein said disease or disorder is an autoimmune disease.

91. The method of claim 90, wherein the autoimmune disease is selected from the group consisting of acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcalnephritis, erythema nodosurn, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, multiple sclerosis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitisubiterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, pamphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis, psoriasis, and fibrosing alveolitis.

92. The method of claim 80, wherein the disease or disorder is selected from the group consisting of myocardial infarction, ischemic heart disease, and atherosclerotic plaques.

93. The method of claim 80, wherein the disease or disorder is graft rejection.

94. The method of claim 80, wherein the disease or disorder is Alzheimer's disease.

95. The method of claim 80, wherein the disease or disorder is caused by atopic tissue.

96. The method of claim 80, wherein the disease or disorder is inflammation caused by accretion of activated granulocytes, monocytes, lymphoid cells or macrophages at the site of inflammation, and wherein the inflammation is caused by an infectious agent.

97. The method of claim 80, wherein said targetable construct is labeled with a radionuclide selected from the group consisting of ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154-158}Gd$, ^{177}LU, ^{32}P, ^{188}Re, and ^{90}Y or a combination thereof.

98. The method of claim 97, wherein said radioactive labels are imaged using computed tomography (CT), single photon emission computed tomography (SPECT), or positron emission tomography (PET).

99. The method of claim 97, wherein the application is for intraoperative diagnosis of the disorder.

100. The method of claim 80, wherein said targetable construct comprises one or more image enhancing agents for use in magnetic resonance imaging (MRI).

101. The method of claim 100, wherein image enhancing agent is a paramagnetic ion selected from the group consisting of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III).

102. The method of claim 80, wherein said targetable construct comprises one or more image enhancing agents for use in ultrasound imaging.

103. The method of claim 102, wherein said targetable construct is a liposome with a **bivalent** HSG-peptide covalently attached to the outside surface of the liposome lipid membrane.

104. The method of claim 103, wherein said liposome is gas filled.

105. A method of antibody dependent enzyme prodrug therapy (ADEPT) comprising; (a) administering to a patient with a neoplastic disorder the polyvalent protein complex of claim 3, wherein said complex comprises a covalently attached enzyme capable of activating a prodrug, (b) optionally administering to said subject a clearing composition, and allowing said composition to clear the polyvalent complex from circulation, and (c) administering said prodrug to the patient.

106. An assay method comprising detecting a target molecule using one or more polyvalent protein complexes of claim 1.

107. An immunostaining method comprising staining a cell using one or more polyvalent protein complexes of claim 1.

108. An isolated nucleic acid molecule encoding a first or second polypeptide according to claim 1.

109. A nucleic acid expression cassette comprising the isolated nucleic acid of claim 108.

110. An episome comprising: (a) a first promoter operationally connected to a first nucleic acid encoding a first polypeptide comprising a polypeptide chain represented by the formula $a_{1\text{-}11\text{-}a2\text{-}12\text{-}a3}$, wherein a_1, a_2, and a_3 are immunoglobulin variable domains and l_1 and l_2 are peptide linkers, (b) a second promoter operationally connected to a

second nucleic acid encoding a polypeptide comprising a second polypeptide chain represented by the formula $b_{1-13-b2-14-b3}$, wherein b_1, b_2, and b_3 are immunoglobulin variable domains and 1_3 and 1_4 are peptide linkers, wherein said first and second polypeptide chain together form a complex comprising at least three antigen binding sites, wherein each of said antigen binding sites comprises a variable domain from said first polypeptide chain and a variable domain from said second polypeptide chain, wherein said first nucleic acid and said second nucleic acid are coexpressed when the episome is transformed into a host cell.

111. The episome of claim 110 which is a plasmid or a cosmid.

112. A host cell comprising an episome according to claim 110.

113. The host cell of claim 112, wherein said host cell is selected from the group consisting of E. coli, yeast, a plant cell and a mammalian cell.

114. A method of preparing a polyvalent protein complex, comprising culturing a host cell according to claim 112.

115. The host cell of claim 112, wherein said cell is a murine myeloma cell line.

116. The episome of claim 111, wherein the plasmid is pdHL2.

L17 ANSWER 2 OF 30 USPATFULL on STN
2004:334260 Subgenomic replicons of the flavivirus dengue.
Pang, Xiaowu, Rockville, MD, UNITED STATES
Dayton, Andrew I., Knoxville, MD, UNITED STATES
Zhang, Mingjie, Bethesda, MD, UNITED STATES
US 2004265338 A1 20041230
APPLICATION: US 2003-656721 A1 20030905 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention discloses the construction of **dengue virus** subgenomic replicons containing large deletions in the structural region (C-preM-E) of the genome, which replicons are useful as vaccines to protect against **dengue virus** infection.

CLM What is claimed is:

1. A subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME).

2. A subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for PreM and E structural proteins (ΔME).

3. A subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for E structural protein (ΔE).

4. A subgenomic replicon of **dengue virus** type 1 origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME).

5. A subgenomic replicon of **dengue virus** type 1 origin comprising a deletion for the sequence coding for PreM and E structural proteins (ΔME).

6. A subgenomic replicon of **dengue virus** type 1 origin comprising a deletion for the sequence coding for E structural protein (ΔE).

7. A subgenomic replicon of **dengue virus** type 2 origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME).

8. A subgenomic replicon of **dengue virus** type 2 origin comprising a deletion for the sequence coding for PreM and E structural proteins (ΔME).

9. A subgenomic replicon of **dengue virus** type 2 origin comprising a deletion for the sequence coding for E structural protein (ΔE).

10. A subgenomic replicon of **dengue virus** type 3 origin comprising a

deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME).

11. A subgenomic replicon of **dengue virus** type 3 origin comprising a deletion for the sequence coding for PreM and E structural proteins (ΔME).

12. A subgenomic replicon of **dengue virus** type 3 origin comprising a deletion for the sequence coding for E structural protein (ΔE).

13. A subgenomic replicon of **dengue virus** type 4 origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME).

14. A subgenomic replicon of **dengue virus** type 4 origin comprising a deletion for the sequence coding for PreM and E structural proteins (ΔME).

15. A subgenomic replicon of **dengue virus** type 4 origin comprising a deletion for the sequence coding for E structural protein (ΔE).

16. A subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE); and further comprising part or all of the 5'UTR; at least about the first 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, or 175 nucleotides of C protein; at least about the last 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, or 175 nucleotides of E protein; substantially all of the nonstructural region; and part or all of the 3'UTR.

17. A subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

18. A vaccine comprising a subgenomic replicon of **dengue virus** origin which comprises a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities, and a pharmaceutically acceptable carrier.

19. A therapeutic comprising a subgenomic replicon of **dengue virus** origin which comprises a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities, and a pharmaceutically acceptable carrier.

20. A **dengue virus** like particle comprising a subgenomic replicon of **dengue virus** origin which comprises a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein

(ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities, and structural proteins of the homologous **dengue virus** wherein said structural proteins encapsulate said subgenomic replicon.

21. A method of immunization comprising administering to an individual in need thereof a subgenomic replicon of **dengue virus** origin which comprises a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

22. A method of immunization comprising administering to an individual in need thereof a **dengue virus** like particle which comprises a subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities, and structural proteins of the homologous **dengue virus** wherein said structural proteins encapsulate said subgenomic replicon.

23. A method of treatment comprising administering to an individual in need thereof a subgenomic replicon of **dengue virus** origin which comprises a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

24. A method of treatment comprising administering to an individual in need thereof a **dengue virus** like particle which comprises a subgenomic replicon of **dengue virus** origin comprising a deletion for the sequence coding for C, PreM, and E structural proteins (ΔCME), for PreM and E structural proteins (ΔME), or for E structural protein (ΔE), optionally which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities, and structural proteins of the homologous **dengue virus** wherein said structural proteins encapsulate said subgenomic replicon.

L17 ANSWER 3 OF 30 USPATFULL on STN
2004:279863 Human glandular kallikrein (hk2)-specific monoclonal antibodies that enhance or inhibit the enzymatic activity of hk2.
Frelinger, John G., Pittsford, NY, UNITED STATES
Fisher, Terrence L., Rochester, NY, UNITED STATES
Nocera, Mary Ann, Bergen, NY, UNITED STATES
Lord, Edith M., Rochester, NY, UNITED STATES
US 2004219163 A1 20041104
APPLICATION: US 2004-491761 A1 20040527 (10)
WO 2002-US31477 20021003
PRIORITY: US 2001-326772P 20011003 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are compositions and methods for preferentially binding hK2 over PSA.
CLM What is claimed is:
1. A composition for generating an antibody response to an antigen, comprising a cell, wherein the cell comprises a vector that encodes the antigen.

2. The composition of claim 1, wherein the cell is derived from a cell line.

3. The composition of claim 2, wherein the cell line is a tumor cell line.

4. The composition of claim 3, wherein the tumor cell line is L1, EMT6, or CT-26.

5. The composition of claim 1, wherein the antigen comprises hK2

6. The composition of claim 1, wherein the antigen is secreted.

7. The composition of claim 1, wherein the antigen is bound to the cell surface.

8. The composition of claim 1, wherein the antigen is internal to the cell.

9. The composition of claim 1, wherein the antigen comprises a peptide or protein produced by a virus.

10. The composition of claim 9, wherein the virus is a Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus virus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6 virus, Human herpes virus 7, Human herpes virus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papilomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, **Dengue virus**, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency cirus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, or Human Immunodeficiency virus type-2.

11. The composition of claim 1, wherein the antigen comprises a peptide or protein produced by a bacterium.

12. The composition of claim 11, wherein the bacterium is a Mycobacterium, Nocardia, Legionella, Salmonella, Shigella, Yersinia, Pasteurella, Actinobacillus, Listeria, Brucella, Cowdria, Chlamydia, Coxiella, Rickettsial, Ehrlichia, Staphylococcus, Streptococcus, Bacillus, Escherichia, Vibrio, Campylobacter, Neiserria, Pseudomonas, Haemophilus, Clostridium, Yersinia.

13. The composition of claim 11, wherein the bacterium is Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis strain BCG, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium africanum, Mycobacterium kansasii, Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium avium, paratuberculosis, Nocardia asteroides, Legionella pneumophila, Salmonella typhi, Shigella, Yersinia pestis, Pasteurella haemolytica, Pasteurella multocida, Actinobacillus pleuropneumoniae, Listeria monocytogenes, Listeria ivanovii, Brucella abortus, Cowdria ruminantium, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci, Coxiella burnetti, Rickettsial, Ehrlichia, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Bacillus anthracis, Escherichia coli, Vibrio cholerae, Campylobacter, Neiserria meningitidis, Neiserria gonorrhea, Pseudomonas aeruginosa, Haemophilus influenzae, Haemophilus ducreyi, Clostridium tetani, Yersinia enterolitica.

14. The composition of claim 1, wherein the antigen is a protein or peptide produced by a parasite.

15. The composition of claim 14, wherein the parasite is a Toxoplasma, Plasmodium, Trypanosoma, Leishmania, Schistosoma, or Entamoeba.

16. The composition of claim 14, wherein the parasite is a Toxoplasma gondii, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Trypanosoma brucei, Trypanosoma cruzi, Leishmania major, Schistosoma mansoni, other Schistosoma, Entamoeba histolytica.

17. The composition of claim 1, wherein the antigen is a protein or peptide produced by a cancer cell.

18. The composition of claim 17, wherein the cancer cell is a Hodgkins lymphoma cell, non-Hodgkins lymphoma cell, B cell lymphoma cell, T cell lymphoma cell, myeloid leukemia cell, leukemias cell, mycosis fungoides cell, carcinoma cell, squamous cell carcinoma cell, adenocarcinoma cell, sarcoma cell, glioma cell, blastoma cell, neuroblastoma cell, plasmacytoma cell, histiocytoma cell, melanoma cell, adenoma cell, hypoxic tumour cell, myeloma cell, AIDS-related lymphoma cell, AIDS related sarcoma cell, metastatic cancer cell, bladder cancer cell, brain cancer cell, nervous system cancer cell, squamous cell carcinoma cell, neuroblastoma cell, glioblastoma cell, ovarian cancer cell, skin cancer cell, liver cancer cell, melanoma cell, colon cancer cell, cervical cancer cell, cervical carcinoma cell, breast cancer cell, epithelial cancer cell, renal cancer cell, genitourinary cancer cell, pulmonary

cancer cell, esophageal carcinoma cell, hematopoietic cancer cell, testicular cancer cell, colo-rectal cancer cell, prostate cancer cell, or pancreatic cancer cell.

19. A composition comprising a substance, wherein the substance preferentially binds hK2 over PSA.

20-27 (canceled)

28. A method of making an antibody comprising injecting an antigen expressing cell into an animal such that the animal has an immune response and isolating antibodies produced by the immune response.

29-59 cancelled

60. A method of screening a sample for the presence of hK2 comprising contacting the sample with a composition that preferentially binds hK2 over PSA.

61-75 cancelled

76. A method of modulating hK2 activity comprising contacting a sample with tion that modulates hK2 activity.

77-101 cancelled

102. A method of treating a subject with a cancer comprising administering to the subject a composition that preferentially binds hK2 over PSA.

L17 ANSWER 4 OF 30 USPATFULL on STN
2004:146867 Recombinant dimeric envelope vaccine against flaviviral infection.
Peters, Iain D., Bozeman, MT, United States
Coller, Beth-Ann G., Woluwe Saint Lambert, BELGIUM
McDonell, Michael, Bogart, GA, United States
Ivy, John M., College Station, TX, United States
Harada, Kent, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6749857 B1 20040615
APPLICATION: US 1999-376463 19990818 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention discloses and claims vaccines containing, as an active ingredient, a secreted recombinantly produced dimeric form of truncated flaviviral envelope protein. The vaccines are capable of eliciting the production of neutralizing antibodies against flaviviruses. The dimeric forms of truncated flaviviral envelope protein are formed 1) by directly linking two tandem copies of 80% E in a head to tail fashion via a flexible tether; 2) via the formation of a leucine zipper domain through the homodimeric association of two leucine zipper helices each fused to the carboxy terminus of an 80% E molecule; or 3) via the formation of a non-covalently associated four-helix bundle domain formed upon association of two helix-turn-helix moieties each attached to the carboxy terminus of an 80% E molecule. All products are expressed as a polyprotein including prM and the modified 80% E products are secreted from Drosophila melanogaster Schneider 2 cells using the human tissue plasminogen activator secretion signal sequence (tPA_L). Secreted products are generally more easily purified than those expressed intracellularly, facilitating vaccine production. One embodiment of the present invention is directed to a vaccine for protection of a subject against infection by **dengue virus**. The vaccine contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue virus** serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional **dengue virus** serotype dimeric E proteins similarly produced. Another embodiment of the present invention is directed to methods to utilize the dimeric form of truncated dengue envelope protein for diagnosis of infection in individuals at risk for the disease. The diagnostic contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue virus** serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The diagnostic may further contain portions of additional **dengue virus** serotype dimeric E proteins similarly produced.
CLM What is claimed is:
1. A vaccine that generates a protective, neutralizing antibody response to a Flavivirus in a murine host, wherein said vaccine comprises a

therapeutically effective amount of a dimeric 80%E, said dimeric 80%E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80%E represents the N-terminal 80% portion of the protein from residue 1 to residue 395.

2. The vaccine of claim 1 wherein said dimeric 80%E is selected from the group consisting of: linked 80%E dimer; 80%E ZipperI; 80%E ZipperII; and 80%E Bundle.

3. The vaccine of claim 2 wherein the linked 80%E dimer is a truncated envelope protein of serotype **DEN**-1.

4. The vaccine of claim 2 wherein the linked 80%E dimer is a truncated envelope protein of serotype **DEN**-2.

5. The vaccine of claim 1 wherein the linked 80%E dimer is a truncated envelope protein of serotype **DEN**-3.

6. The vaccine of claim 1 wherein the linked 80%E dimer is a truncated envelope protein of serotype **DEN**-4.

7. A **multivalent** vaccine that generates a protective, neutralizing antibody response to a Flavivirus in a murine host, wherein said vaccine comprises a therapeutically effective amount of a first dimeric 80%E product of one flaviviral serotype; a second dimeric 80%E product of a second flaviviral serotype; a third dimeric 80%E product of a third flaviral serotype; and a fourth dimeric 80%E product of a fourth flaviviral serotype; wherein all dimeric 80%E products have been secreted as recombinantly produced protein from a Drosophila Schneider cell, wherein 80%E is the N-terminal 80% of the protein from residue 1 to 395.

8. The vaccine of claim 7 wherein said dimeric 80%E products are envelope proteins of serotypes selected from the group consisting of: **DEN**-1; **DEN**-2; **DEN**-3; and **DEN**-4.

9. The vaccine of claim 1 wherein said Flavivirus is a **dengue virus**.

10. The vaccine of claim 2 wherein said Flavivirus is a **dengue virus**.

11. The vaccine of claim 7 wherein said Flavivirus is a **dengue virus**.

12. An immunogenic polypeptide comprising a dimeric 80%E, said dimeric 80%E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80%E represents the N-terminal 80% of the protein from residue 1 to residue 395.

13. The immunogenic polypeptide of claim 12 wherein said dimeric 80%E is selected from the group consisting of: linked 80%E dimer, 80%E ZipperI; 80%E ZipperII; and 80%E bundle.

14. The immunogenic polypeptide of claim 13 wherein the linked 80%E dimer is a truncated envelope protein which is at least one member selected from the group consisting of serotype **DEN**-1, serotype **DEN**-2, serotype **DEN**-3, and serotype **DEN**-4.

15. An immunogenic composition that generates a protective, neutralizing antibody response to a Flavivirus in a murine host, comprising the immunogenic polypeptide defined in claim 12 and a physiologically acceptable carrier.

16. The immunogenic composition defined in claim 15 further comprising an adjuvant.

17. The immunogenic composition defined in claim 15 wherein said adjuvant is Iscomatrix.

18. The immunodiagnostic for the detection of Flavivirus comprising the immunogenic polypeptide defined in claim 12.

19. A **multivalent** immunodiagnostic for the detection of Flavivirus comprising at least two of the immunogenic polypeptides defined in claim 12 of at least two flaviviral serotypes.

20. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the immunogenic polypeptide defined in claim 12; b) a suitable support phase coated with dimeric 80%E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

21. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the **multivalent** immunodiagnostic polypeptide defined in claim 19; b) a suitable support phase coated with dimeric 80%E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

L17 ANSWER 5 OF 30 USPATFULL on STN
2004:38218 Use of phyllanthus component for the treatment or prophylaxis of infections triggered by flaviviridae.
Von Keudell, Christoph, Ammerland, GERMANY, FEDERAL REPUBLIC OF
van der Giessen, Robert H.M., Aesch, SWITZERLAND
US 2004028754 A1 20040212
APPLICATION: US 2003-311961 A1 20030725 (10)
WO 2001-EP6981 20010620
PRIORITY: DE 2000-10030139 20000620
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the use of one or more Phyllanthus components or substances or mixtures of substances obtained therefrom for preventing or treating infectious diseases that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof. Furthermore, the invention relates to the use of one or more Phyllanthus components or substances or mixtures of substances obtained therefrom for producing a pharmaceutical composition for the prevention or treatment of infectious diseases that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof. The invention also relates to the use of one or more Phyllanthus components or substances or mixtures of substances obtained therefrom for inhibiting the propagation of viruses belonging to the family of Flaviviridae. Moreover, the invention relates to a method for preventing or treating infectious diseases in a mammal that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof, wherein one or more Phyllanthus components or substances or mixtures of substances obtained therefrom are administered to the mammal. In addition, the invention relates to a method for inhibiting the propagation of viruses belonging to the family of Flaviviridae, wherein one or more Phyllanthus components or substances or mixtures of substances obtained therefrom are contacted with the viruses. The hepatitis C virus is a preferred virus.

CLM What is claimed is:

1. Use of one or more Phyllanthus component(s) or substances or mixtures of substances obtained therefrom for preventing or treating infectious diseases that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof.

2. Use of one or more Phyllanthus component(s) or substances or mixtures of substances obtained therefrom for producing a pharmaceutical composition, medicinal preparation or medicinal adjuvant for preventing or treating infectious diseases that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof.

3. The use according to claim 1 or 2, wherein the infectious diseases comprise yellow fever, **dengue** fever, Central European tick-borne fever, cirrhosis, hepatocellular carcinoma, membranoproliferative glomerulonephritis.

4. Use of one or more Phyllanthus component(s) or substances or mixtures of substances obtained therefrom for inhibiting the propagation of viruses belonging to the family of Flaviviridae.

5. The use according to claim 4, wherein the inhibition takes place ex vivo or in vitro.

6. The use according to any one of claims 1 to 5, wherein the one or more Phyllanthus components or substances or mixtures of substances obtained therefrom are derived from Phyllanthus amarus, Phyllanthus niruri, Phyllanthus emblica, Phyllanthus urinaria, Phyllanthus acidus, Phyllanthus acuminatus and Phyllanthus reticulatus or from several of these Phyllanthus species.

7. The use according to any one of claims 1 to 6, wherein the viruses of the family of Flaviviridae belong to the genus flavivirus.

8. The use according to claim 7, wherein the genus flavivirus comprises the group of **dengue viruses**, the Japanese encephalitis viruses, the modoc viruses, the mosquito-borne viruses, the Ntaya viruses, the R10 Bravo viruses, the tick-borne viruses, the Tyuleniy viruses, the Uganda-S viruses, the yellow fever viruses and the non-classified flaviviruses, in particular the Central European tick-borne fever flavivirus.

9. The use according to any one of claims 1 to 6, wherein the viruses of the family of Flaviviridae belong to the genus of hepatitis C-like viruses.

10. The use according to claim 9, wherein the genus of hepatitis C-like viruses comprises the hepatitis C virus.

11. The use according to any one of claims 1 to 6, wherein the viruses of the family of Flaviviridae belong to the genus of pestivirus.

12. The use according to claim 11, wherein the genus of pestivirus comprises the group of bovine viral diarrhea virus-2, the pestivirus giraffe H138, the pestiviruses strain giraffe-1, the pestivirus type 1, the pestivirus type 2, in particular the hog cholera virus, and the pestivirus type 3.

13. The use according to any one of claims 1 to 6, wherein the viruses of the family of Flaviviridae belong to the non-classified flaviviruses.

14. The use according to claim 13, wherein the non-classified flaviviruses comprise douroucouli hepatitis GB virus A, GBV-A-like virus, the group of GBV-C/HG viruses, in particular the hepatitis G virus, hepatitis GB virus A, hepatitis GB virus B, the marmoset hepatitis GB virus A, mosquito cell fusing agent and turkey meningoencephalitis virus.

15. The use according to any one of claims 1 to 14, wherein the prevention or treatment comprises the inhibition of one or more viral enzymes.

16. The use according to claim 15, wherein the inhibition comprises the inhibition of the processing of one or more viral enzymes.

17. The use according to claim 15 or 16, wherein the viral enzymes are selected from the group consisting of NS2A, NS2B, NS3, NS4A, NS5, NS5A and NS5B.

18. The use according to any one of claims 1 to 17, wherein the Phyllanthus components comprise, herba drug, leaves, cortex, flowering, seeds, fruits, stalk, branches, stem, roots and wood.

19. The use according to any one of claims 1 to 18, wherein the substances or mixtures of substances are selected from the group consisting of alkaloids, tannins, gallotannins, lignans, sesquiterpenes, triterpenes, proteins, peptides, polysaccharides, glycosides, **glycoproteins**, flavonoids, sterols and caffeic acid esters.

20. The use according to claim 19, wherein alcohols and/or mixtures thereof are used for extracting the substance or mixture of substances.

21. The use according to claim 20, wherein the alcohols used are short-chain primary C1 to C4 alcohols and/or mixtures thereof.

22. The use according to claim 21, wherein the alcohols used are methanol and/or ethanol.

23. The use according to any one of claims 1 to 22, wherein one or more of the Phyllanthus components or the substances or mixtures or substances obtained therefrom are used in combination with one or more other active agents.

24. The use according to claim 23, wherein the other active agents are selected from the group of cytokines.

25. The use according to claim 24, wherein the group of cytokines comprises immune modulators, in particular interferon α, interferon β, interferon γ and interleukins.

26. The use according to claim 23, wherein the other active agents are selected from the group of the nucleoside analogues.

27. The use according to claim 26, wherein the group of the nucleoside analogues comprises ribavirin, adefovir dipivoxir, famciclovir and FTC.

28. The use according to claim 23, wherein the other active agents are selected from the group of antibodies.

29. The use according to any one of claims 1 to 28, wherein the application is in form of an infusion solution, an injection solution, oral forms of application, an ointment, a therapeutic pack, a granulate, food supplements or in form of clysters.

30. The use according to any one of claims 1 to 29, wherein the application takes place orally, topically or parenterally.

31. A method for preventing or treating infectious diseases in a mammal that are triggered by viruses belonging to the family of Flaviviridae or infectious diseases in which viruses belonging to the family of Flaviviridae are involved in the development or progression thereof, wherein one or more Phyllanthus component(s) or substances or mixtures of substances obtained therefrom are administered to the mammal.

32. The method according to claim 31, wherein the mammal is a human.

33. A method for inhibiting the propagation of viruses belonging to the family of Flaviviridae, wherein one or more Phyllanthus component(s) or substances or mixtures of substances obtained therefrom are contacted with the viruses.

34. The method according to claim 33, wherein the inhibition takes place ex vivo or in vitro.

35. The method according to any one of claims 31 to 34, wherein the virus is the hepatitis C virus (HCV).

L17 ANSWER 6 OF 30 USPATFULL on STN
2004:30623 Methods of using Flt3-ligand in immunization protocols.
McKenna, Hilary J., Seattle, WA, UNITED STATES
Liebowitz, David N., Seattle, WA, UNITED STATES
Maliszewski, Charles R., Seattle, WA, UNITED STATES
US 2004022760 A1 20040205
APPLICATION: US 2003-401364 A1 20030326 (10)
PRIORITY: US 2002-368263P 20020326 (60)
US 2002-427835P 20021119 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of using Flt3-ligand (Flt3-L) in immunization protocols to enhance immune responses against vaccine antigens. Embodiments include administering Flt3-ligand prior to immunizing a subject with a vaccine, wherein the vaccine comprises at least one antigen formulated in one or more adjuvants. Methods of treating and preventing disease and infection using Flt3-ligand immunization protocols are also provided. Methods of using Flt3-ligand immunization protocols for in vivo evaluation of antigens and adjuvants are also provided.

CLM What is claimed is:

1. A method of immunizing a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises an antigen and an adjuvant.

2. The method of claim 1, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

3. The method of claim 1, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

4. The method of claim 1, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

5. The method of claim 1, wherein the adjuvant is selected from the group consisting of ADJUMER.TM. (polyphosphazene); aluminum phosphate gel; algal glucans; algammulin; aluminum hydroxide gel (alum); high protein adsorbency aluminum hydroxide gel; low viscosity aluminum hydroxide gel; AF or SPT (emulsion of squalane (5%), Tween 80(0.2%), Pluronic L121(1.25%), phosphate-buffered saline pH 7.4); AVRIDE.TM. (propanediamine); BAY R1005.TM. ((N-(2-Deoxy-2-L-leucylamino-b-D-glucopyranosyl)-N-octadecyldodecanoylamide hydroacetate); CALCITRIOL.TM. (1α, 25-dihydroxyvitamin D3); calcium phosphate gel; CAP.TM. (calcium phosphate nanoparticles); cholera holotoxin, cholera toxin Al-protein A-D fragment fusion protein, cholera toxin B subunit; CRL 1005 (Block Copolymer P1205); cytokine containing liposomes; DDA (dimethyldioctadecylammonium bromide); DHEA (dehydroepiandrosterone); DMPC (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); DOC/Alum Complex (deoxycholic Acid Sodium Salt); Freund's Complete Adjuvant; Freund's Incomplete Adjuvant; Gamma Inulin; Gerbu Adjuvant (mixture of: i) N-Acetylglucosaminyl-(Pl-4)-N-acetylmuramyl-L-alanyl-D-glutamine (GMDP), ii) Dirnethyl dioctadecylammonium. chloride (DDA), iii) Zinc L-proline salt complex (ZnPro-8); GM-CSF; GMDP (N-acetylglucosaminyl-(b1-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine); Imiquimod (1-(2-methypropyl)-IH-imidazo[4,5-c]quinolin-4-amine); ImmTher.TM. (N-acetylglucosaminyl-N-acetyhnuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate); DRVs (Immunoliposomes prepared from Dehydration-Rehyrdation Vesicles); Interferon-γ; Interleukin-1β; Interleukin-2; Interleukin-7; Interleukin-12; ISCOMS.TM. (Immune Stimulating Complexes); ISCOPREP 7.0.3. .TM.; Liposomes; LOXORIBINE.TM. (7-allyl-8-oxoguanosine); LT Oral Adjuvant.TM. (E. coli labile enterotoxin protoxin); Microspheres and Microparticles of any composition; MF59.TM.; (squalene.water emulsion); MONTANIDE ISA 51.TM. (purified Incomplete Freund's Adjuvant); MONTANIDE ISA 720.TM. (metabolizable oil adjuvant); MPL.TM. (3-Q-desacyl-4'-monophosphoryl lipid A); MTP-PE and MTP-PE liposomes ((N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxy-phosphoryloxy)) ethylamide, mono sodium salt); MURAMETIDE.TM. (Nac-Mur-L-Ala-D-Gln-OCH3); MURAPALMITINE.TM. and D-MURAPALMITINE.TM. (Nac-Mur-L-Thr-D-isoGIn-sn-glycerol dipalmitoyl); NAGO (Neuraminidase-galactose oxidase); Nanospheres or Nanoparticles of any composition; NISVs (Non-Ionic Surfactant Vesicles); PLEURAN.TM. (β-glucan); PLGA, PGA and PLA (homo-and co-polymers of lactic and glycolic acid; micro-/nanospheres); PLURONIC L121.TM.; PMMA (polymethyl methacrylate); PODDS.TM. (oroteinoid microspheres); Polyethylene carbamate derivatives; Poly rA:Poly rU (Poly-adenylic acid-poly-uridylic acid complex); Polysorbate 80 (Tween 80); Protein Cochleates (Avanti Polar Lipids, Inc., Alabaster, Ala.); STIMULON.TM. (QS-21); Quil-A (Quil-A saponin); S-28463 (4-Amino-otec,-dimethyl-2-ethoxymethyl-1H-imidazo[4,5-c]quinoline-1-ethanol); SAF-1.TM. (Syntex Adjuvant Formulation); Sendai proteoliposomes and Sendai-containing lipid matrices; Span-85 (sorbitan trioleate); Specol (emulstion of Marcol 52, Span 85 and Tween 85); Squalene or Robane® (2,6,10,15,19,23-hexamethyltetracosane and 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22 tetracosahexaene); Stearyl Tyrosine (Octadecyl tyrosine hydrochloride); Theramide® (N-acetylglucosaminyl-N-acetylinuramyl-L-Ala-D-isoGlu-L-Ala-dipalmitoxy propylamide); Theronyl-MDP (Termurtide.TM. or [thr 1]-MDP; N-acetyl muramyl-L-threonyl-D-isoglutamine); Ty Particles (Ty-VLPs or virus like particles); Walter Reed Liposomes

6. The method of claim 1, wherein the antigen is a cancer antigen.

7. The method of claim 6, wherein the cancer antigen is selected from the group consisting of Melanoma-Melanocyte Differentiation Antigens (MART-1/Melan A; gp100/pmel-17; Tyrosinase; Tyronsinase Related Protein-1; Tyronsinase Related Protein-2; Melanocyte-Stimulating Hormone Receptor); Cancer-Testes Antigens (MAGE-1; MAGE-2; MAGE-3; MAGE-12, BAGE; CAGE, NYESO-1); Mutated Antigens (β-catenin; MUM-1; CDK-4; Caspase-8; KIA 0205; HLA-A2-R1701); and Non-Mutated Shared Antigens Overexpressed on Cancers (α-Fetoprotein; Telomerase Catalytic Protein; G-250; MUC-1; Carcinoembryonic antigen; p53; Her-2/neu), epitopes from Non-Mutated Proteins (gp100; MAGE-1; MAGE-3; Tyrosinase; NY-ESO-1) and epitopes from Mutated Proteins (Triosephosphate isomerase; CDC-27; LDLR-FUT).

8. The method of claim 1, wherein the antigen is a viral antigen.

9. The method of claim 8, wherein the viral antigen is selected from the group consisting of Retroviridae (e.g., human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g., polio viruses, hepatitis A virus; enteroviruses, human coxsackie viruses,

rhinoviruses, echoviruses); Calciviridae (e.g., strains that cause gastroenteritis); Togaviridae (e.g., equine encephalitis viruses, rubella viruses); Flaviridae (e.g., **dengue viruses**, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g., coronaviruses); Rhabdoviridae (e.g., vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g., ebola viruses); Paramyxoviridae (e.g., parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g., influenza viruses); Bunyaviridae (e.g., Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g., reoviruses, orbiviuises and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvovirusies); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes viruses'); Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g., African swine fever virus); viral agents of non-A, non-B hepatitis; Norwalk and related viruses, and astroviruses).

10. The method of claim 1, wherein the antigen is a bacterial antigen.

11. The method of claim 10, wherein the bacterial antigen is selected from the group consisting of Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria sps (e.g. M. tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae), Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes (Group A Streptococcus), Streptococcus agalactiae (Group B Streptococcus), Streptococcus (viridans group), Streptococcus faecalis, Streptococcus bovis, Streptococcus (anaerobic sps.), Streptococcus pneumoniae, pathogenic Campylobacter sp., Enterococcus sp., Haemophilus influenzae, Bacillus antracis, Corynebacterium diphtheriae, corynebacterium sp., Erysipelothrix rhusiopathiae, Clostridium perfringers, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Bacteroides sp., Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, and Actinomyces israelli.

12. The method of claim 1, wherein the antigen is from an infectious unicellular organism.

13. The method of claim 12, wherein the antigen is selected from the group consisting of schistosomes; trypanosomes; Leishmania species; filarial nematodes; trichomoniasis; sarcosporidiasis; Taenia saginata, Taenia solium, Cryptococcus neoformans, Apergillus fumigatus, Histoplasma capsulatum, Coccidiodes immitis, trichinelosis, Blastomyces dermatitidis, Chlamydia trachomatis, Candida albicans, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Toxoplasma gondii.

14. A method of treating cancer in a subject having cancer, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises a cancer antigen and an adjuvant.

15. The method of claim 14, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

16. The method of claim 14, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

17. The method of claim 14, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-IBB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

18. A method of preventing and/or treating viral infection in a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises a viral antigen and an adjuvant.

19. The method of claim 18, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

20. The method of claim 18, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

21. The method of claim 18, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

22. A method of preventing and/or treating bacterial infection in a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises a bacterial antigen and an adjuvant.

23. The method of claim 22, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

24. The method of claim 22, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

25. The method of claim 22, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

26. A method of enhancing an immune response to an antigen in a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises an antigen and an adjuvant.

27. The method of claim 26, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

28. The method of claim 26, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

29. The method of claim 26, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

30. A method of enhancing an antigen-specific cytotoxic T-cell immune response to an antigen in a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises an antigen and an adjuvant.

31. The method of claim 30, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

32. The method of claim 30, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

33. The method of claim 31, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-IBB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

34. A method of enhancing an antigen-specific T-helper immune response to an antigen in a subject, comprising the steps of: (a) administering

Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; and, (c) administering a vaccine to the subject, wherein the vaccine comprises an antigen and an adjuvant.

35. The method of claim 34, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

36. The method of claim 34, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

37. The method of claim 34, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

38. A method of evaluating the immune responses to an antigen in a subject, comprising the steps of: (a) administering Flt3-ligand to a subject; (b) optionally administering an auxiliary molecule; (c) administering an antigen to the subject, wherein the antigen may optionally be formulated with an adjuvant; and, (d) evaluating the subject's immune responses to the antigen.

39. The method of claim 38, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

40. The method of claim 38, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the vaccine.

41. The method of claim 38, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

42. A method of treating allgeries in a subject having one or more allergies, comprising the steps of: (a) administering Flt3-ligand to the subject; (b) optionally administering an auxiliary molecule; and, (c) administering an allergy vaccine to the subject.

43. The method of claim 42, wherein Flt3-ligand is administered prior to, concurrent with and/or subsequent to administration of the allergy vaccine.

44. The method of claim 42, wherein the auxiliary molecule is administered prior to, concurrent with and/or subsequent to administration of the allergy vaccine.

45. The method of claim 42, wherein the auxiliary molecule is selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12, 15, 18 and 23, chemokines, GM-CSF, G-CSF, Interferon-alpha and gamma, c-kit ligand, fusions of GM-CSF and IL-3, TNF family members (TNF-, TGF-β, soluble CD40 ligand, CD40-binding proteins, soluble CD83, 4-1BB binding proteins, OX-40 binding proteins, CpG sequences, and combinations thereof.

L17 ANSWER 7 OF 30 USPATFULL on STN
2004:12660 Immunotherapeutics for biodefense.
Bowdish, Katherine S., Del Mar, CA, UNITED STATES
Frederickson, Shana, Solana Beach, CA, UNITED STATES
Wild, Martha A., San Diego, CA, UNITED STATES
Maruyama, Toshiaki, La Jolla, CA, UNITED STATES
Nolan, Mary Jean, San Diego, CA, UNITED STATES
US 2004009178 A1 20040115
APPLICATION: US 2003-364743 A1 20030211 (10)
PRIORITY: US 2002-356086P 20020211 (60)
US 2002-376408P 20020429 (60)
US 2002-414053P 20020927 (60)
US 2002-428807P 20021125 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Human neutralizing antibodies (full-length or functional fragments) are useful as anti-toxins or anti-infectives with respect to infective agents such as, for example, anthrax, botulinum, smallpox, Venezuelan equine encephalomyelitis virus (VEEV), West Nile virus (WNV) and the like.

CLM What is claimed is:

1. A human heterodimeric antibody or antibody fragment having a binding affinity of at least 1×10^{-8} M to the protective antigen of Bacillus anthracis and the ability to block binding of the protective antigen to one or more members of the group consisting of cell receptors, edema factor and lethal factor.

2. A human heterodimeric antibody or antibody fragment having a binding affinity of at least 1×10^{-8}M to a molecule involved in anthrax infection and the ability to block binding of said molecule involved in anthrax infection to one or more members of the group consisting of cell receptors, PA63, PA63 heptamer, PA83, edema factor and lethal factor.

3. A human heterodimeric antibody or antibody fragment as in claim 1 that prevents PA63 from forming a heptamer.

4. A human heterodimeric antibody or antibody fragment as in claim 1 that prevents PA63 from binding to EF or LF.

5. A human heterodimeric antibody or antibody fragment as in claim 1 that prevents EF and/or LF from binding to the PA63 heptamer.

6. A human heterodimeric antibody or antibody fragment comprising a heavy chain variable region having a sequence selected from the group consisting of SEQ ID NO. 1 to 18.

7. A human heterodimeric antibody or antibody fragment comprising a light chain kappa region having a sequence selected from the group consisting of SEQ ID NO. 19 to 26.

8. A human heterodimeric antibody or antibody fragment comprising a light chain lambda region having a sequence selected from the group consisting of SEQ ID NO. 27 to 38.

9. A human heterodimeric antibody or antibody fragment comprising a light chain kappa region having a sequence selected from the group consisting of SEQ ID NO. 39 to 61.

10. A human heterodimeric antibody or antibody fragment comprising a light chain lambda region having a sequence selected from the group consisting of SEQ ID NO. 62 to 77.

11. A human heterodimeric antibody or antibody fragment comprising a heavy chain variable region having a sequence selected from the group consisting of SEQ ID NO. 78 to 112.

12. A method of screening antibodies comprising: preparing a combinatorial library using RNA isolated from cells obtained from a human subject producing antibodies against one or more molecules involved in anthrax infection; and screening the combinatorial library for an antibody having a binding affinity of at least 1×10^{-8} M to a molecule involved in anthrax infection and the ability to block binding of said molecule involved in anthrax infection to one or more members of the group consisting of cell receptors, PA63, PA63 heptamer, PA83 edema factor and lethal factor.

13. A method comprising: preparing a combinatorial library using RNA isolated from cells obtained from a human subject that has been vaccinated against or exposed to a plurality of infective agents; and screening the combinatorial library for a plurality of antibodies each having a binding affinity to a molecule involved in infection by at least one infective agent.

14. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an antibody having a binding affinity of at least 1×10^{-8} M to a molecule involved in anthrax infection and the ability to block binding of said molecule involved in anthrax infection to one or more members of the group consisting of cell receptors, PA63, PA63 heptamer, PA83, edema factor and lethal factor.

15. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an anti-Venezuelan equine

encephalomyelitis virus antibody.

16. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an anti-botulinum antibody.

17. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an anti-West Nile virus antibody.

18. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an anti-orthopox antibody.

19. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is an anti-**dengue** antibody.

20. A method as in claim 13 wherein the step of preparing a combinatorial library comprises using RNA isolated from cells obtained from a human subject that has been vaccinated against or exposed to one or more infective agents selected from the group consisting of anthrax, botulinum, smallpox, **dengue**, Venezuelan equine encephalomyelitis virus and West Nile virus.

21. A method as in claim 13 wherein the step of preparing a combinatorial library comprises using RNA isolated from cells obtained from a human subject that has been vaccinated against or exposed to two or more infective agents selected from the group consisting of anthrax, botulinum, smallpox, **dengue**, Venezuelan equine encephalomyelitis virus and West Nile virus.

22. A method as in claim 13 wherein one of the plurality of antibodies identified in the step of screening is selected from the group consisting of anti-variola antibodies, anti-monkeypox virus antibodies and anti-vaccinia virus antibodies.

23. A human heterodimeric antibody or antibody fragment having a binding affinity to a molecule involved in Venezuelan equine encephalomyelitis virus infection and the ability to neutralize Venezuelan equine encephalomyelitis virus.

24. A human heterodimeric antibody or antibody fragment comprising a heavy chain comprising a sequence selected from the group consisting of SEQ ID NO. 116 to 118.

25. A human heterodimeric antibody or antibody fragment comprising a light chain comprising a sequence selected from the group consisting of SEQ ID NO. 113 to 115.

L17 ANSWER 8 OF 30 USPATFULL on STN
2004:1830 Antigen library immunization.
Punnonen, Juha, Belmont, CA, UNITED STATES
Bass, Steven H., Hillsborough, CA, UNITED STATES
Whalen, Robert Gerald, Foster City, CA, UNITED STATES
Howard, Russell, Los Altos Hills, CA, UNITED STATES
Stemmer, Willem P.C., Los Gatos, CA, UNITED STATES
Maxygen, Inc., a Delaware corporation (U.S. corporation)
US 2004001849 A1 20040101
APPLICATION: US 2003-383317 A1 20030307 (10)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention is directed to antigen library immunization, which provides methods for obtaining antigens having improved properties for therapeutic and other uses. The methods are useful for obtaining improved antigens that can induce an immune response against pathogens, cancer, and other conditions, as well as antigens that are effective in modulating allergy, inflammatory and autoimmune diseases.
CLM What is claimed is:
1. An recombinant **multivalent** antigenic polypeptide that comprises a first antigenic determinant of a first polypeptide and at least a second antigenic determinant from a second polypeptide.

2. The **multivalent** antigenic polypeptide of claim 1, wherein the polypeptide comprises at least a third antigenic determinant from a third polypeptide.

3. The **multivalent** antigenic polypeptide of claim 1, wherein the first and second polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens

associated with inflammatory conditions, antigens associated with allergic reactions, and antigens from infectious agents.

4. The **multivalent** antigenic polypeptide of claim 3, wherein the antigens are from a virus, a parasite, or a bacteria.

5. The **multivalent** antigenic polypeptide of claim 4, wherein the antigens are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a **Dengue virus**, a Hanta virus, an HIV, a hepatitis B virus, a hepatitis C virus, and a Herpes simplex virus.

6. The **multivalent** antigenic polypeptide of claim 5, wherein the antigens are envelope proteins.

7. The **multivalent** antigenic polypeptide of claim 4, wherein the antigens are from a bacteria and are selected from the group consisting of a Yersinia V antigen, a Staphylococcus aureus enterotoxin, a Streptococcits pyogenes enterotoxin, a Vibrio cholera toxin, an enterotoxigenic Escherichia coli heat labile enterotoxin, a OspA and a OspC polypeptide from a Borrelia species, an Antigen 85 polypeptide from a Mycobacterium species, a VacA and a CagA polypeptide from Helicobacter pylori, and an MSP antigen from Plasmodium falciparum.

8. The **multivalent** antigenic polypeptide of claim 1, wherein the **multivalent** antigenic polypeptide exhibits reduced affinity to IgE from a mammal compared to the first or second polypeptides.

9. The **multivalent** antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different serotypes of a pathogenic organism.

10. The **multivalent** antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different species of pathogenic organism.

11. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are allergens.

12. The **multivalent** antigenic polypeptide of claim 11, wherein the allergens are dust mite allergens, grass pollen allergens, birch pollen allergens, ragweed pollen allergens, hazel pollen allergens, cockroach allergens, rice allergens, olive tree pollen allergens, fungal allergens, mustard allergens, and bee venom.

13. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are associated with an inflammatory or autoimmune disease.

14. The **multivalent** antigenic polypeptide of claim 13, wherein the first polypeptide and the second polypeptide are autoantigens associated with a disease selected from the group consisting of multiple sclerosis, scleroderma, systemic sclerosis, systemic lupus erythematosus, hepatic autoimmune disorder, skin autoimmune disorder, insulin-dependent diabetes mellitus, thyroid autoimmune disorder, and rheumatoid arthritis.

15. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are cancer antigens or sperm antigens.

16. A recombinant antigen library comprising recombinant nucleic acids that encode antigenic polypeptides, wherein the library is obtained by recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes a disease-associated antigenic polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids.

17. The recombinant antigen library of claim 16, wherein the first and second polypeptides are toxins.

18. A method of obtaining a polynucleotide that encodes a recombinant antigen having improved ability to induce an immune response to a disease condition, the method comprising: (1) recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes an antigenic polypeptide that is associated with the disease condition, wherein the first and second

forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library to identify at least one optimized recombinant nucleic acid that encodes an optimized recombinant antigenic polypeptide that has improved ability to induce an immune response to the disease condition.

19. The method of claim 18, wherein the method further comprises: (3) recombining at least one optimized recombinant nucleic acid with a farther form of the nucleic acid, which is the same or different from the first and second forms, to produce a further library of recombinant nucleic acids; (4) screening the further library to identify at least one further optimized recombinant nucleic acid that encodes a polypeptide that has improved ability to induce an immune response to the disease condition; and (5) repeating (3) and (4), as necessary, until the further optimized recombinant nucleic acid encodes a polypeptide that has improved ability to induce an immune response to the disease condition.

20. The method of claim 18, wherein the disease-associated polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens associated with inflammatory conditions, antigens associated with allergic reactions, and antigens associated with infectious agents.

21. The method of claim 18, wherein the disease condition is an infectious disease and the first and second forms of the nucleic acid each encode an antigen of a different serotype of a pathogenic agent.

22. The method of claim 21, wherein the first and second forms of the nucleic acid are each from a different species of pathogen.

23. The method of claim 21, wherein the screening is accomplished by: introducing into a test animal either: a) the library of recombinant nucleic acids, or b) recombinant polypeptides encoded by the library of recombinant nucleic acids; introducing the pathogenic agent into the test animal; and determining whether the test animal is resistant to challenge by the pathogenic agent.

24. The method of claim 23, wherein the pathogenic agent introduced into the test animal is of a different serotype than that used as a source of the first and second forms of the nucleic acid.

25. The method of claim 23, wherein the library is subdivided into a plurality of pools, each of which pools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

26. The method of claim 25, wherein the pools that include an optimized recombinant nucleic acid are further subdivided into a plurality of subpools, each of which subpools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

27. The method of claim 18, wherein the optimized recombinant nucleic acid encodes a **multivalent** antigenic polypeptide and the screening is accomplished by: expressing the library of recombinant nucleic acids in a phage display expression vector such that the recombinant antigen is expressed as a fusion protein with a phage polypeptide that is displayed on a phage particle surface; contacting the phage with a first antibody that is specific for a first serotype of the pathogenic agent and selecting those phage that bind to the first antibody; contacting those phage that bind to the first antibody with a second antibody that is specific for a second serotype of the pathogenic agent and selecting those phage that bind to the second antibody; wherein those phage that bind to the first antibody and the second antibody express a **multivalent** antigenic polypeptide.

28. The method of claim 27, wherein the screening further comprises contacting those phage that bind to the first and second antibodies with one or more additional antibodies, each of which is specific for an additional serotype of the pathogenic agent, and selecting those phage that bind to the respective additional antibodies.

29. The method of claim 27, wherein the phage display expression vector comprises a suppressible stop codon between the recombinant nucleic acid and the phage polypeptide, whereby expression in a host cell which comprises a corresponding suppressor tRNA results in production of the

fusion protein and expression in a host cell which lacks a corresponding suppressor tRNA results in production of the recombinant antigen not as a fusion protein.

30. The method of claim 18, wherein the optimized recombinant antigen exhibits an enhanced expression level in a host cell and the screening is accomplished by expression of each recombinant nucleic acid in the host cell and subjecting the host cells to flow cytometry-based cell sorting to obtain those host cells that display the recombinant antigen on the host cell surface.

31. The method of claim 18, wherein the improved property is selected from the group consisting of: improved immunogenicity; enhanced cross-reactivity against different forms of the disease-associated antigenic polypeptide; reduced toxicity; improved adjuvant activity in vivo; and improved production of the immunogenic polypeptide.

32. The method of claim 31, wherein the improved property is enhanced cross-reactivity against different forms of the disease-associated polypeptide and the first and second forms of the nucleic acid are from a first and a second form of the disease-associated polypeptide.

33. The method of claim 32, wherein the first and second forms of the disease-associated polypeptide are obtained from at least a first and second species of a pathogenic agent and the optimized recombinant nucleic acid encodes a recombinant polypeptide that induces a protective response against both species of the pathogenic agent.

34. The method of claim 33, wherein the recombinant polypeptide induces a protective response against at least one additional species of the pathogenic agent.

35. The method of claim 33, wherein the pathogenic agent is a toxin.

36. The method of claim 33, wherein the pathogenic agent is a virus or a cell.

37. The method of claim 33, wherein the disease-associated polypeptide is a Yersinia V-antigen.

38. The method of claim 37, wherein the at least first and second forms of a nucleic acid are obtained from at least a first and second species of Yersinia.

39. The method of claim 38, wherein the Yersinia species are selected from the group consisting of Y. pestis, Y. enterocolitica, and Y. pseudotuberculosis.

40. The method of claim 33, wherein the pathogenic agent is a bacterial toxin.

41. The method of claim 18, wherein the disease condition is cancer and the screening step involves introducing the optimized recombinant nucleic acids into a genetic vaccine vector and testing library members for ability to inhibit proliferation of cancer cells or inducing death of cancer cells.

42. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a tumor specific antigen.

43. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a molecule which is capable of inhibiting proliferation of cancer cells.

44. The method of claim 18, wherein the disease condition is an inflammatory response which has an unknown or no antigen specificity and the screening step involves one or more of the following: a) determining the ability of the genetic vaccine vector to induce cytokine production by PBMC, synovial fluid cells, purified T cells, monocytes/macrophages, dendritic cells, or T cell clones; b) determining the ability of the genetic vaccine vector to induce T cell activation or proliferation; and c) determining the ability of the genetic vaccine vector to induce T cell differentiation to T_{H1} or T_{H2} cells.

45. The method of claim 18, wherein the disease condition is an autoimmune response.

46. The method of claim 45, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a $T_{H1\text{-mediated}}$ response to a $T_{H2\text{-mediated}}$ response.

47. The method of claim 18, wherein the disease condition is an allergic immune response.

48. The method of claim 47, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a $T_{H2\text{-mediated}}$ response to a $T_{H1\text{-mediated}}$ response.

49. The method of claim 47, wherein the optimized recombinant antigenic polypeptide induces an immune response characterized by predominant IgG and IgM expression and reduced IgE expression.

50. The method of claim 47, wherein the optimized recombinant antigenic polypeptide is not recognized by pre-existing IgE molecules present in sera of atopic mammals.

51. The method of claim 50, wherein the optimized recombinant antigenic polypeptide retains T cell epitopes that are involved in modulating a T cell response.

52. A method of obtaining a recombinant viral vector which has an enhanced ability to induce an antiviral response in a cell, the method comprising the steps of: (1) recombining at least first and second forms of a nucleic acid which comprise a viral vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant viral vectors; (2) transfecting the library of recombinant viral vectors into a population of mammalian cells; (3) staining the cells for the presence of Ix protein; and (4) isolating recombinant viral vectors from cells which stain positive for Mx protein, wherein recombinant viral vectors from positive staining cells exhibit enhanced ability to induce an antiviral response.

53. The method of claim 52, wherein the viral vector comprises an influenza viral genomic nucleic acid.

L17 ANSWER 9 OF 30 USPATFULL on STN
2003:318275 Recombinant Newcastle disease viruses useful as vaccines or vaccine vectors.
Samal, Siba K., College Park, MD, UNITED STATES
Huang, Zhuhui, College Park, MD, UNITED STATES
US 2003224017 A1 20031204
APPLICATION: US 2003-440419 A1 20030519 (10)
PRIORITY: US 2002-381462P 20020517 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns an antigenomic RNA of Newcastle Disease virus (NDV) carrying one or more foreign genes inserted before NP gene, between P and M genes, and/or between HN and L genes. The invention is also directed toward a cDNA encoding a recombinant antigenomic RNA having one or more foreign genes inserted according to the invention, a cell containing the cDNA, a plasmid comprising the cDNA, a cell containing the plasmid, a cell containing the recombinant antigenomic RNA, and a recombinant NDV containing the recombinant antigenomic RNA of the invention, such as a recombinant NDV carrying one or more foreign genes recovered from transcription of the cDNA or the plasmid in a competent cell. The recombinant NDV carrying the one or more foreign genes can be used as a vaccine or vaccine vector.
CLM What is claimed is:
1. An antigenomic RNA of Newcastle disease virus, comprising NP gene, P gene, M gene, F gene, HN gene and L gene in this order from a 5' to 3' direction, said antigenomic RNA further comprising n foreign nucleotide complexes inserted (a) before the NP gene, (b) between the P and M genes, and/or (c) between the HN and L genes, wherein n is 1, 2, 3 or 4; each of the foreign nucleotide complexes comprising a Newcastle disease virus gene start sequence, an open reading frame of a foreign gene and a Newcastle disease virus gene end sequence in this order from the 5' to 3' direction, wherein the foreign gene is a gene not found naturally in the Newcastle disease virus; and wherein when 2, 3 or 4 foreign nucleotide complexes are inserted together before the NP gene, between the P and M genes, or between the HN and L genes, the foreign nucleotide complexes are sequentially linked directly or indirectly.

2. The antigenome RNA of claim 1, wherein n is 2, 3 or 4 and the foreign nucleotide complexes are different.

3. The antigenome RNA of claim 1, wherein n is 2, 3 or 4 and the foreign nucleotide complexes are the same.

4. The antigenome RNA of claim 1, wherein n is 1 or 2.

5. The antigenome RNA of claim 4, wherein n is 2 and the foreign nucleotide complexes are different.

6. The antigenome RNA of claim 4, wherein n is 2 and the foreign nucleotide complexes are the same.

7. The antigenome RNA of claim 1, wherein the length of the open reading frame of the foreign gene is no more than about 3000 nucleotides.

8. The antigenome RNA of claim 7, wherein the length of the open reading frame of the foreign gene is no more than about 1500 nucleotides.

9. The antigenome RNA of claim 8, wherein the length of the open reading frame of the foreign gene is no more than about 1000 nucleotides.

10. The antigenome RNA of claim 9, wherein the length of the open reading frame of the foreign gene is no more than about 800 nucleotides.

11. The antigenome RNA of claim 10, wherein the length of the open reading frame of the foreign gene is no more than about 500 nucleotides.

12. The antigenome RNA of claim 11, wherein the length of the open reading frame of the foreign gene is no more than about 300 nucleotides.

13. The antigenome RNA of claim 1, wherein 2, 3 or 4 foreign nucleotide complexes are inserted together before the NP gene, between the P and M genes, or between the HN and L genes, the sequentially linked foreign nucleotide complexes having a combined length of no more than about 5000 nucleotides.

14. The antigenomic RNA of claim 13, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 2000 nucleotides.

15. The antigenomic RNA of claim 14, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 1000 nucleotides.

16. The antigenomic RNA of claim 15, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 800 nucleotides.

17. The antigenomic RNA of claim 1, wherein the foreign genes of the foreign nucleotide complexes are selected from the group consisting of genes encoding chloramphenical acetyltransferase, green fluorescent protein, an avian cytokine, and an immunogenic protein of a virus selected from the group consisting of influenza virus, infectious bursal disease virus, rotavirus, infectious bronchitis virus, infectious laryngotracheitis virus, chicken anemia virus, Marek's disease virus, avian leukosis virus, avian adenovirus, and avian pneumovirus.

18. The antigenomic RNA of claim 1, wherein the foreign genes of the foreign nucleotide complexes encode chloramphenical acetyltransferase.

19. The antigenomic RNA of claim 1, wherein the foreign genes of the foreign nucleotide complexes encode the same or different avian cytokines.

20. The antigenomic RNA of claim 19, wherein the avian cytokines are avian interleukins.

21. The antigenomic RNA of claim 20, wherein the avian cytokines are avian IL-2 and/or IL-4.

22. The antigenomic RNA of claim 1, wherein the foreign genes of the foreign nucleotide complexes encode an immunogenic protein of the same or different viruses selected from the group consisting of influenza virus, infectious bursal disease virus, rotavirus, infectious bronchitis virus, infectious laryngotracheitis virus, chicken anemia virus, Marek's disease virus, avian leukosis virus, avian adenovirus and avian pneumovirus.

23. The antigenomic RNA of claim 1, wherein the n foreign nucleotide complexes are inserted (a) before the NP gene, and/or (b) between the P

and M genes.

24. The antigenomic RNA of claim 23, wherein the n foreign nucleotide complexes are inserted before the NP gene.

25. The antigenome RNA of claim 24, wherein 2, 3 or 4 foreign nucleotide complexes are inserted together before the NP gene, the sequentially linked foreign nucleotide complexes having a combined length of no more than about 4000 nucleotides.

26. The antigenomic RNA of claim 25, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 2000 nucleotides.

27. The antigenomic RNA of claim 26, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 1000 nucleotides.

28. The antigenomic RNA of claim 27, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 800 nucleotides.

29. The antigenomic RNA of claim 23, wherein the n foreign nucleotide complexes are inserted between the P and M genes.

30. The antigenomic RNA of claim 29, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 4000 nucleotides.

31. The antigenomic RNA of claim 30, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 2000 nucleotides.

32. The antigenomic RNA of claim 31, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 1000 nucleotides.

33. The antigenomic RNA of claim 32, wherein the sequentially linked foreign nucleotide complexes have a combined length of no more than about 800 nucleotides.

34. The antigenomic RNA of claim 18, wherein n is 1.

35. The antigenomic RNA of claim 19, wherein n is 1.

36. The antigenomic RNA of claim 22, wherein n is 1.

37. The antigenomic RNA of claim 24, wherein n is 1.

38. The antigenomic RNA of claim 37, wherein the open reading frame of the foreign gene encodes chloramphenicol acetyltransferase.

39. The antigenomic RNA of claim 37, wherein the open reading frame of the foreign gene encodes an avian cytokine.

40. The antigenomic RNA of claim 39, wherein the avian cytokine is an avian interleukin.

41. The antigenomic RNA of claim 40, wherein the avian interleukin is IL-2 or IL-4.

42. The antigenomic RNA of claim 1, having a length of m nucleotides, wherein m is a multiple of 6.

43. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes contain foreign gene encoding an immunogenic protein of a non-avian pathogen.

44. The antigenomic RNA of claim 43, wherein the non-avian pathogen is a virus selected from the group consisting of influenza virus, SARS-causing virus, human respiratory syncytial virus, human immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, rabies virus, Hendra virus, Nipah virus, human parainfluenza 3 virus, measles virus, mumps virus, Ebola virus, Marburg virus, West Nile virus, Japanese encephalitis virus, **Dengue virus**, Hantavirus, Rift Valley fever virus, Lassa fever virus, herpes simplex virus and yellow fever virus.

45. A cDNA encoding the antigenomic RNA of claim 1.

46. A plasmid comprising the cDNA of claim 45.

47. A cell comprising the cDNA of claim 45.

48. A cell comprising the plasmid of claim 46.

49. A cell comprising the antigenomic RNA of claim 1.

50. A recombinant Newcastle disease virus produced by a process comprising the following steps: (i) providing cells capable of synthesizing T7 RNA polymerase; (ii) transfecting the cells with a plasmid comprising the cDNA encoding the antigenomic RNA of claim 1, a plasmid encoding NP protein, a plasmid encoding P protein, and a plasmid encoding L protein to obtain transfected cells in a medium; and thereafter (iii) isolating Newcastle disease virus from a supernatant of the medium of step (ii) to obtain the recombinant Newcastle disease virus.

51. The recombinant Newcastle disease virus of claim 50, wherein the cells capable of synthesizing T7 RNA polymerase provided in step (i) are from a cell line expressing T7 RNA polymerase.

52. The recombinant Newcastle disease virus of claim 50, wherein the cells capable of synthesizing T7 RNA polymerase provided in step (i) are plant cells.

53. The recombinant Newcastle disease virus of claim 50, wherein the cells capable of synthesizing T7 RNA polymerase provided in step (i) are mammalian cells.

54. The recombinant Newcastle disease virus of claim 50, wherein the cells capable of synthesizing T7 RNA polymerase provided in step (i) are avian cells.

55. The recombinant Newcastle disease virus of claim 50, wherein the cells capable of synthesizing T7 RNA polymerase provided in step (i) are HEp-2 cells infected with a virus that can synthesize T7 RNA polymerase.

56. The recombinant Newcastle disease virus of claim 55, wherein the virus is a vaccinia virus.

57. A recombinant Newcastle disease virus produced by a process comprising the following steps: (i) providing cells capable of synthesizing T7 RNA polymerase; (ii) transfecting the cells with a plasmid comprising the cDNA encoding the antigenomic RNA of claim 18, a plasmid encoding NP protein, a plasmid encoding P protein, and a plasmid encoding L protein to obtain transfected cells in a medium; and thereafter (iii) isolating Newcastle disease virus from a supernatant of the medium of step (ii) to obtain the recombinant Newcastle disease virus.

58. A recombinant Newcastle disease virus produced by a process comprising the following steps: (i) providing cells capable of synthesizing T7 RNA polymerase; (ii) transfecting the cells with a plasmid comprising the cDNA encoding the antigenomic RNA of claim 19, a plasmid encoding NP protein, a plasmid encoding P protein, and a plasmid encoding L protein to obtain transfected cells in a medium; and thereafter (iii) isolating Newcastle disease virus from a supernatant of the medium of step (ii) to obtain the recombinant Newcastle disease virus.

59. A recombinant Newcastle disease virus produced by a process comprising the following steps: (i) providing cells capable of synthesizing T7 RNA polymerase; (ii) transfecting the cells with a plasmid comprising the cDNA encoding the antigenomic RNA of claim 22, a plasmid encoding NP protein, a plasmid encoding P protein, and a plasmid encoding L protein to obtain transfected cells in a medium; and thereafter (iii) isolating Newcastle disease virus from a supernatant of the medium of step (ii) to obtain the recombinant Newcastle disease virus.

60. A recombinant Newcastle disease virus produced by a process comprising the following steps: (i) providing cells capable of synthesizing T7 RNA polymerase; (ii) transfecting the cells with a plasmid comprising the cDNA encoding the antigenomic RNA of claim 43, a plasmid encoding NP protein, a plasmid encoding P protein, and a plasmid encoding L protein to obtain transfected cells in a medium; and thereafter (iii) isolating Newcastle disease virus from a supernatant

of the medium of step (ii) to obtain the recombinant Newcastle disease virus.

61. A method of vaccinating an avian animal against Newcastle disease, wherein the avian animal is in need of the vaccination, comprising administering an effective amount of the recombinant Newcastle disease virus of claim 50 to the avian animal.

62. A method of vaccinating an avian animal against Newcastle disease, wherein the avian animal is in need of the vaccination, comprising administering an effective amount of the recombinant Newcastle disease virus of claim 57 to the avian animal.

63. A method of treating an avian animal with an avian cytokine, wherein the avian animal is in need of the treatment, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 58 to the avian animal.

64. A method of immunizing an avian animal against an avian pathogen selected from the group consisting of influenza virus, infectious bursal disease virus, rotavirus, infectious bronchitis virus, infectious laryngotracheitis virus, chicken anemia virus, Marek's disease virus, avian Leukosis virus, avian adenovirus and avian pneumovirus, wherein the avian animal is in need of the immunization, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 59 to the avian animal, wherein the open reading frame of the foreign gene encodes an immunogenic protein of the avian pathogen against which the avian animal is immunized.

65. A method of immunizing a mammal against a non-avian pathogen, wherein the mammal is in need of the immunization, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 60 to the mammal, wherein the open reading frame of the foreign gene encodes an immunogenic protein of the non-avian pathogen against which the mammal is immunized.

66. The method of claim 65, wherein the non-avian pathogen is selected from the group consisting of influenza virus, SARS-causing virus, human respiratory syncytial virus, human immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, rabies virus, Hendra virus, Nipah virus, human parainfluenza 3 virus, measles virus, mumps virus, Ebola virus, Marburg virus, West Nile virus, Japanese encephalitis virus, **Dengue virus**, Hantavirus, Rift Valley fever virus, Lassa fever virus, herpes simplex virus and yellow fever virus.

67. A process of making the antigenomic RNA of claim 1, comprising the following steps: (i) providing a cDNA comprising NP gene, P gene, M gene, F gene, HN gene and L gene in this order, said cDNA further comprising n foreign nucleotide complexes inserted (a) before the NP gene, (b) between the P and M genes, and/or (c) between the HN and L genes, wherein n is 1, 2, 3 or 4; each of the foreign nucleotide complexes comprising a Newcastle disease virus gene start sequence, an open reading frame of a foreign gene and a Newcastle disease virus gene end sequence in this order from the 5' to 3' direction, wherein the foreign gene is a gene not found naturally in the Newcastle disease virus; wherein when n is 2, 3 or 4, the foreign nucleotide complexes are the same or different; and wherein when 2, 3 or 4 foreign nucleotide complexes are inserted together before the NP gene, between the P and M genes, or between the HN and L genes, the foreign nucleotide complexes are sequentially linked directly or indirectly; (ii) transcribing the cDNA to form a mixture containing an antigenomic RNA; and thereafter (iii) isolating the antigenomic RNA.

68. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes is inserted before the NP gene.

69. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes is inserted before the NP gene and at least one of the foreign nucleotide complexes is inserted between the P and M genes.

70. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes is inserted before the NP gene and at least one of the foreign nucleotide complexes is inserted between the HN and L genes.

71. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes is inserted before the NP gene, at least one of the foreign nucleotide complexes is inserted between the P and M genes, and at least one of the foreign nucleotide complexes is inserted between the HN and L genes.

72. The antigenomic RNA of claim 1, wherein at least one of the foreign nucleotide complexes is inserted between the P and M genes.

73. The antigenomic RNA of claim 1, further comprising at least one intergenic region selected from the group consisting of a NP-P intergenic region between the NP and P genes, a P-M intergenic region between the P and M genes, a M-F intergenic region between the M and F genes, a F-HN intergenic region between the F and HN genes, and a HN-L intergenic region between the HN and L genes.

74. The antigenomic RNA of claim 1, further comprising a NP-P intergenic region between the NP and P genes, a P-M intergenic region between the P and M genes, a M-F intergenic region between the M and F genes, a F-HN intergenic region between the F and HN genes, and a HN-L intergenic region between the HN and L genes.

75. The antigenomic RNA of claim 1, wherein the foreign gene of at least one of the foreign nucleotide complexes encodes a tumor antigen.

76. The antigenomic RNA of claim 75, wherein the tumor antigen is selected from the group consisting of pg100, MAGE1, MAGE3 and CDK4.

77. A recombinant Newcastle disease virus comprising the antigenomic RNA of claim 1.

78. A recombinant Newcastle disease virus comprising the antigenomic RNA of claim 18.

79. A recombinant Newcastle disease virus comprising the antigenomic RNA of claim 19.

80. A recombinant Newcastle disease virus comprising the antigenomic RNA of claim 22.

81. A recombinant Newcastle disease virus comprising the antigenomic RNA of claim 43.

82. A method of vaccinating an avian animal against Newcastle disease, wherein the avian animal is in need of the vaccination, comprising administering an effective amount of the recombinant Newcastle disease virus of claim 77 to the avian animal.

83. A method of vaccinating an avian animal against Newcastle disease, wherein the avian animal is in need of the vaccination, comprising administering an effective amount of the recombinant Newcastle disease virus of claim 78 to the avian animal.

84. A method of treating an avian animal with an avian cytokine, wherein the avian animal is in need of the treatment, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 79 to the avian animal.

85. A method of immunizing an avian animal against an avian pathogen selected from the group consisting of influenza virus, infectious bursal disease virus, rotavirus, infectious bronchitis virus, infectious laryngotracheitis virus, chicken anemia virus, Marek's disease virus, avian Leukosis virus, avian adenovirus and avian pneumovirus, wherein the avian animal is in need of the immunization, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 80 to the avian animal, wherein the open reading frame of the foreign gene encodes an immunogenic protein of the avian pathogen against which the avian animal is immunized.

86. A method of immunizing a mammal against a non-avian pathogen, wherein the mammal is in need of the immunization, said method comprising administering an effective amount of the recombinant Newcastle disease virus of claim 81 to the mammal, wherein the open reading frame of the foreign gene encodes an immunogenic protein of the non-avian pathogen against which the mammal is immunized.

87. The antigenomic RNA of claim 1, wherein at least one foreign nucleotide complex is inserted between the P and M genes and at least one foreign nucleotide complex is inserted before the NP gene.

88. The antigenomic RNA of claim 1, wherein at least one foreign nucleotide complex is inserted between the P and M genes and at least one foreign nucleotide complex is inserted between the HN and L genes.

L17 ANSWER 10 OF 30 USPATFULL on STN
2003:282308 HBV core antigen particles with multiple immunogenic components attached via peptide ligands.
Murray, Kenneth, Edinburgh, UNITED KINGDOM
Biogen, Inc. (non-U.S. corporation)
US 2003198649 A1 20031023
APPLICATION: US 2003-448546 A1 20030529 (10)
PRIORITY: US 1998-110911P 19981204 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to hepatitis B virus ("HBV") core antigen particles that are characterized by multiple immunogen specificities. More particularly, the invention relates to HBV core antigen particles comprising immunogens, epitopes, or other related structures, crosslinked thereto by ligands which are HBV capsid-binding peptides that selectively bind to HBV core protein. Such particles may be used as delivery systems for a diverse range of immunogenic epitopes, including the HBV capsid-binding peptides, which advantageously also inhibit and interfere with HBV viral assembly by blocking the interaction between HBV core protein and HBV surface proteins. Mixtures of different immunogens and/or capsid-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or **multivalent** HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compositions, as well as in diagnostic compositions and methods using them.

CLM What is claimed is:

1. An HBV core antigen particle comprising at least one capsid binding immunogen, said capsid binding immunogen comprising at least one HBV capsid-binding peptide component and at least one immunogenic component.

2. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen is oriented on said particle such that it permits said immunogenic component to elicit an immune response when said particle is administered to an individual.

3. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen is linked to said particle through any amino acid residue of said HBV capsid-binding peptide component.

4. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen is linked to said particle through any amino acid residue or other residue of said immunogenic component.

5. The HBV core antigen particle according to claim 4, wherein said other residue of said immunogenic component is a carbohydrate.

6. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen is linked to said particle through the amino terminus of said HBV capsid-binding peptide component.

7. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen is linked to said particle through the carboxy terminus of said HBV capsid-binding peptide component.

8. The HBV core antigen particle according claim 1, wherein said capsid binding immunogen is crosslinked to said particle by a crosslinker.

9. The HBV core antigen particle according to claim 1, wherein said immunogenic component is linked to said HBV capsid-binding peptide component directly or through a linker sequence.

10. The HBV core antigen particle according to claim 1, wherein said immunogenic component is linked to the amino terminus of said HBV capsid-binding peptide component directly or through a linker sequence.

11. The HBV core antigen particle according to claim 1, wherein said immunogenic component is linked to the carboxy terminus of said HBV capsid-binding peptide component directly or through a linker sequence.

12. The HBV core antigen particle according to any one of claims 9-11, wherein said immunogenic component is linked to said HBV capsid-binding peptide component by a crosslinker.

13. The HBV core antigen particle according to claim 8, wherein said crosslinker is a multifunctional crosslinker.

14. The HBV core antigen particle according to claim 12, wherein said crosslinker is a multifunctional crosslinker.

15. The HBV core antigen particle according to claim 14, wherein said multifunctional crosslinker is selected from the group consisting of 1-ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride and N-hydroxy-sulphosuccinimide.

16. The HBV core antigen particle according to claim 1, wherein said immunogenic component comprises one or more epitopes selected from the group consisting of immunologic epitopes, immunogenic epitopes and antigenic epitopes.

17. The HBV core antigen particle according to claim 16, wherein said epitopes are selected from the group consisting of linear epitopes, conformational epitopes, single epitopes and mixed epitopes.

18. The HBV core antigen particle according to claim 1, wherein said immunogenic component is selected from the group consisting of antigens, allergens, antigenic determinants, proteins, **glycoproteins**, antibodies, antibody fragments, peptides, peptide mimotopes which mimic an antigen or antigenic determinant, polypeptides, glycopeptides, carbohydrates, oligosaccharides, polysaccharides, oligonucleotides and polynucleotides.

19. The HBV core antigen particle according to claim 1, wherein said immunogenic component is targeted to or derived from a pathogenic agent selected from the group consisting of viruses, parasites, mycobacteria, bacteria, bacilli, fungi, protozoa, plants, phage, animal cells and plant cells.

20. The HBV core antigen particle according to claim 19, wherein said virus is selected from the group consisting of retroviruses, herpesviruses, orthomyoxoviruses, paramyxoviruses, hepadnaviruses, flaviviruses, picornaviruses, papoviruses, adenoviruses, baculoviruses, hantaviruses, parvoviruses, enteroviruses, rhinoviruses, tumor viruses, DNA viruses, RNA viruses, togaviruses, rhabdoviruses and poxviruses.

21. The HBV core antigen particle according to claim 20, wherein said virus is selected from the group consisting of human immunodeficiency type 1 virus, human immunodeficiency type 2 virus, T cell-leukemia virus, herpes simplex type 1 virus, herpes simplex type 2 virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, influenza A virus, influenza B virus, influenza C virus, respiratory syncytial virus, measles-like virus, mumps virus, parainfluenza virus, hepatitis B virus, hepatitis C virus, hepatitis A virus, hepatitis E virus, yellow fever virus, malaria, **dengue virus**, tick-borne encephalitis virus, oncovirus, poliomyelitis virus, papillomavirus, rubella virus, rabies virus and vaccinia virus.

22. The HBV core antigen particle according to claim 19, wherein said immunogenic component is targeted to or derived from bacillus, enterobacteria, clostridium, listeria, mycobacterium, pseudomonas, staphylococcus, eubacteria, mycoplasma, chlamydia, spirochetes, neisseria or salmonella.

23. The HBV core antigen particle according to claim 19, wherein said immunogenic component is targeted to diptheria, tetanus, acellular pertussis, haemophilus influenza, polio, measles, mumps, rubella, varicella, hepatitis B virus, hepatitis A virus, pneumococcal pneumonia, yellow fever, malaria, hepatitis B virus, hepatitis A virus, typhoid fever, meningococcal encephalitis or cholera.

24. The HBV core antigen particle according to claim 18, wherein said immunogenic component is selected from the group consisting of animal allergens, insect allergens, plant allergens, atmospheric allergens and inhalant allergens.

25. The HBV core antigen particle according to claim 1, wherein said HBV core antigen is an HBV core antigen fusion protein.

26. The HBV core antigen particle according to claim 25, wherein said HBV core antigen fusion protein comprises an immunologic epitope, an immunogenic epitope or an antigenic epitope.

27. The HBV core antigen particle according to claim 26, wherein said HBV core antigen fusion protein comprises an immunologic epitope, an immunogenic epitope or an antigenic epitope fused to HBV core antigen directly or through a linker sequence.

28. The HBV core antigen particle according to claim 26, wherein said

HBV core antigen fusion protein comprises an immunologic epitope, an immunogenic epitope or an antigenic epitope fused to the carboxy terminus of said HBV core antigen directly or through a linker sequence.

29. The HBV core antigen particle according to claim 26, wherein said HBV core antigen fusion protein comprises an immunologic epitope, an immunogenic epitope or an antigenic epitope fused to the amino terminus of said HBV core antigen directly or through a linker sequence.

30. The HBV core antigen particle according to claim 25, wherein said HBV core antigen fusion protein comprises truncated HBV core antigen.

31. The HBV core antigen particle according to claim 25, wherein said HBV core antigen fusion protein comprises HBV surface antigen or portions thereof.

32. The HBV core antigen particle according to claim 31, wherein said HBV core antigen fusion protein comprises a sequence selected from the group consisting of the pre-S1 region of HBV surface antigen, the pre-S2 region of HBV surface antigen, the immunodominant a region of HBV surface antigen and portions thereof.

33. The HBV core antigen particle according to claim 1, wherein said HBV core antigen is a full length HBV core antigen polypeptide, or portions, truncates, mutations or derivatives thereof which are capable of assembling in particulate form.

34. The HBV core antigen particle according to claim 1, wherein said HBV capsid-binding peptide component is selected from the group consisting of: SLLGRMKGA, GSLLGRMKGA, DGSLLGRMKGAA, ADGSLLGRMKGAAG, SLLGRMKG(β-A)C, RSLLGRMKGA, HRSLLGRMKGA, ALLGRMKG, MHRSLLGRMKGA, RSLLGRMKGA(β-A)C and MHRSLLGRMKGAG(β-A)GC.

35. A vaccine comprising a prophylactically effective amount of an HBV core antigen particle according to claim 1.

36. A pharmaceutical composition comprising a therapeutically effective amount of an HBV core antigen particle according to claim 1.

37. A method for producing an immune response in an individual comprising the step of administering to said individual an HBV core antigen particle according to claim 1 in an amount effective to produce an immune response.

38. The method according to claim 37, wherein said HBV core antigen particle is administered to said individual by parenteral route.

39. A method for increasing the immunogencity of an immunogen by linking said immunogen to an HBV core antigen particle through an HBV capsid-binding peptide.

40. The HBV core antigen particle according to claim 1, wherein said capsid binding immunogen comprises a diagnostic label or a chemical marker.

41. A method for detecting the presence of antibodies to an immunogen in a sample comprising the steps of: (a) contacting the sample with an HBV core antigen particle according to claim 40, for a time sufficient to permit any antibodies in said sample to form a complex with said capsid binding immunogen and; (b) using detection means to detect the complex formed between the capsid binding immunogen and said antibodies in said sample.

42. An HBV capsid-binding peptide immunogen comprising at least one capsid binding peptide component and at least one immunogenic component.

43. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is linked to said HBV capsid-binding peptide directly or through a linker sequence.

44. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is linked to the amino terminus of said HBV capsid-binding peptide component directly or through a linker sequence.

45. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is linked to the carboxy terminus of said HBV capsid-binding peptide component directly or through a linker sequence.

46. The HBV capsid-binding peptide immunogen according to any one of claims 42-44, wherein said immunogenic component is crosslinked to said HBV capsid-binding peptide component by a crosslinker.

47. The HBV capsid-binding peptide immunogen according to claim 46, wherein said crosslinker is a multifunctional crosslinker.

48. The HBV capsid-binding peptide immunogen according to claim 47, wherein said multifunctional crosslinker is selected from the group consisting of 1-ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride and N-hydroxy-sulphosuccinimide.

49. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component comprises one or more epitopes selected from the group consisting of immunologic epitopes, immunogenic epitopes and antigenic epitopes.

50. The HBV capsid-binding peptide immunogen according to claim 49, wherein said epitopes are selected from the group consisting of linear epitopes, conformational epitopes, single epitopes and mixed epitopes.

51. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is selected from the group consisting of antigens, allergens, antigenic determinants, proteins, **glycoproteins**, antibodies, antibody fragments, peptides, peptide mimotopes which mimic an antigen or antigenic determinant, polypeptides, glycopeptides, carbohydrates, oligosaccharides, polysaccharides, oligonucleotides and polynucleotides.

52. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is targeted to or derived from a pathogenic agent selected from the group consisting of viruses, parasites, mycobacteria, bacteria, bacilli, fungi, protozoa, plants, phage, animal cells and plant cells.

53. The HBV capsid-binding peptide immunogen according to claim 52, wherein said virus is selected from the group consisting of retroviruses, herpesviruses, orthomyoxoviruses, paramyxoviruses, hepadnaviruses, flaviviruses, picornaviruses, papoviruses, adenoviruses, baculoviruses, hantaviruses, parvoviruses, enteroviruses, rhinoviruses, tumor viruses, DNA viruses, RNA viruses, togaviruses, rhabdoviruses and poxviruses.

54. The HBV capsid-binding peptide immunogen according to claim 53, wherein said virus is selected from the group consisting of human immunodeficiency type 1 virus, human immunodeficiency type 2 virus, T cell-leukemia virus, herpes simplex type 1 virus, herpes simplex type 2 virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, influenza A virus, influenza B virus and influenza C virus, respiratory syncytial virus, measles-like virus, mumps virus, parainfluenza virus, hepatitis B virus, hepatitis C virus, hepatitis A virus, hepatitis E virus, yellow fever virus, **dengue virus**, malaria, tick-borne encephalitis virus, poliomyelitis virus, rubella virus, rabies virus and vaccinia virus.

55. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is targeted to or derived from bacillus, enterobacteria, clostridium, listeria, mycobacterium, pseudomonas, staphylococcus, eubacteria, mycoplasma, chlamydia, spirochetes, neisseria or salmonella.

56. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is targeted to diptheria, tetanus, acellular pertussis, haemophilus influenza, polio, measles, mumps, rubella, varicella, hepatitis B virus, hepatitis A virus, pneumococcal pneumonia, yellow fever, malaria, hepatitis B virus, hepatitis A virus, typhoid fever, meningococcal encephalitis or cholera.

57. The HBV capsid-binding peptide immunogen according to claim 42, wherein said immunogenic component is selected from the group consisting of animal allergens, insect allergens, plant allergens, atmospheric allergens and inhalant allergens.

58. The HBV capsid-binding peptide immunogen according to claim 42, wherein said HBV capsid-binding peptide component is selected from the group consisting of: SLLGRMKGA, GSLLGRMKGA, DGSLLGRMKGAA, ADGSLLGRMKGAAG, SLLGRMKG(β-A)C, RSLLGRMKGA, HRSLLGRMKGA, ALLGRMKG, MHRSLLGRMKGA, RSLLGRMKGA(β-A)C and MHRSLLGRMKGAG(β-A)GC.

L17 ANSWER 11 OF 30 USPATFULL on STN
2003:276776 Use of flavivirus for the expression of protein epitopes and development of new live attenuated vaccine virus to immune against flavivirus and other infectious agents.
Bonaldo, Mirna C., Rio de Janeiro, BRAZIL
Galler, Ricardo, Rio de Janeiro, BRAZIL
Freire, Marcos da Silva, Rio de Janeiro, BRAZIL
Garrat, Richard C., Sao Paulo, BRAZIL
US 2003194801 A1 20031016
APPLICATION: US 2003-275707 A1 20030410 (10)
WO 2002-BR36 20020308
PRIORITY: GB 2001-5877 20010309
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a vaccine against infections caused by flavivirus. More particularly to the use of the YF vaccine virus (17D) to express at the level of its envelope, protein epitopes from other pathogens which will elicit a specific immune response to the parental pathogen.
CLM What is claimed is:

1. A method for the production of Flavivirus as a vector for heterologous antigens comprising the introduction and expression of foreign gene sequences into insertion sites at the level of the envelope protein of any Flavivirus, wherein the sites are structurally apart from areas known to interfere with the overall flavivirus E protein structure and comprising: (i) sites that lie on the external surface of the virus providing accessibility to antibody; (ii) not disrupt or significantly destabilize the three-dimensional structure of the E protein; and, (iii) not interfere with the formation of the E protein network within the viral envelope.

2. The method according to claim 1 wherein one site comprises the region of β-strands f and g including the fg loop which form part of the five-stranded anti-parallel β-sheet of domain II of the flavivirus envelope protein.

3. The method according to claim 2 wherein the site is the loop area between β-strands f and g which form part of the five-stranded anti-parallel β-sheet of domain II of the flavivirus envelope protein.

4. The method according to claim 2 wherein the foreign sequence has been inserted in the region of amino acid 196 to 215 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

5. The method according to claim 3 wherein the foreign sequence has been inserted in the region of amino acid 205 to 210 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

6. The method according to claim 1 wherein another site comprises the region of E_0 and F_0 strands including the E_{0F0} loop which form part of the eight stranded β-barrel of domain I.

7. The method according to claim 6 wherein the site is the loop area between E_0 and F_0 strands which form part of the eight stranded β-barrel of domain I.

8. The method according to claim 6 wherein the foreign sequence has been inserted in the region of amino acid 138 to 166 with reference to the tick-borne encephalitis virus sequence described in FIG. 2

9. The method according to claim 7 wherein the foreign sequence has been inserted in the region of amino acid 146 to 160 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

10. The method according to claim 1 wherein the Flavivirus is selected from the group consisting of any Flavivirus including yellow fever virus, tick borne encephalitis virus, **dengue virus** and japanese encephalitis virus.

11. The method according to claim 10 wherein the virus is a wild type, attenuated or recombinant virus.

12. The method according to claims 10 and 11 wherein the Flavivirus is a yellow fever virus.

13. The method according to claim 12 wherein the virus is a recombinant yellow fever virus.

14. The method according to claim 13 wherein the yellow fever virus is the YF17D virus strain and substrains thereof.

15. The method according to claims 1 to 14 wherein the foreign epitope is a malarial gene sequence.

16. The method according to claim 15 wherein the malarial gene sequence is the $(NANP)_3$ humoral epitope.

17. The method according to claim 15 wherein the malarial gene sequence is the DYENDIEKKI cytotoxic T-lymphocytes (CTL) epitope.

18. The method according to claim 15 wherein the malarial gene sequence is the SYVPSAEQI cytotoxic T-lymphocytes (CTL) epitope.

19. The method according to claim 1 wherein one or more glycine residues is inserted in the region immediately upstream and downstream of the foreign epitope.

20. A DNA construct consisting essentially of a vector, a genetically stable Flavivirus genome and foreign gene sequences introduced in an insertion site according to any of claims 1 to 19.

21. The DNA construct according to claim 20 wherein the Flavivirus is selected from the group consisting of any Flavivirus including yellow fever virus, tick borne encephalitis virus, **dengue virus** and japanese encephalitis virus

22. The DNA construct according to claim 20 wherein the vector is selected from the group consisting of low copy number plasmids.

23. The DNA construct according to claim 21 wherein the vector is selected from the group consisting of pACNR1180 and pBeloBAC11.

24. The DNA construct according to claim 20 wherein the vector is selected from the group consisting of high copy number plasmids.

25. The DNA construct according to claim 20 wherein the genetically stable Flavivirus genome is derived from any YF 17D strain.

26. The DNA construct according to claim 25 wherein the genetically stable Flavivirus genome is the YF genome bearing the complete sequence set forth in SEQ ID NO:1 or functionally equivalent sequences thereof.

27. The DNA construct according to claim 20 wherein the foreign gene sequence is derived from malaria, yellow fever, **dengue**, Japanese encephalitis, tick-borne encephalitis and fungi infections.

28. The DNA construct according to claim 27 wherein the foreign gene sequence is a malarial gene sequence.

29. The DNA construct according to claims 26 and 28 wherein the malarial gene sequence is the $(NANP)_3$ humoral epitope.

30. The DNA construct according to claim 28 wherein the malarial gene sequence is the DYENDIEKKI cytotoxic T-lymphocytes epitope.

31. The DNA construct according to claim 28 wherein the malarial gene sequence is the SYVPSAEQI cytotoxic T-lymphocytes epitope.

32. The DNA construct according to claim 29 which is plasmid pYF17D/8.

33. DNA construct having the structure of plasmid pYFE200.

34. DNA construct having the structure of plasmid pYFE200/1.

35. DNA construct having the structure of plasmid pYFE200/13.

36. DNA construct having the structure of plasmid pYFE200/8.

37. A Flavivirus as a vector for heterologous antigens comprising foreign gene sequences inserted at sites in the level of its envelope protein, wherein the sites are structurally apart from areas known to interfere with the overall flavivirus E protein structure.

38. The Flavivirus according to claim 37 wherein the foreign gene

sequence is introduced in the region of β-strands f and g including the fg loop which form part of the five-stranded anti-parallel β-sheet of domain II of the flavivirus envelope protein.

39. The Flavivirus according to claim 38 wherein the site is the loop area between β-strands f and g which form part of the five-stranded anti-parallel β-sheet of domain II of the flavivirus envelope protein.

40. The Flavivirus according to claim 38 wherein the foreign sequence has been inserted in the region of amino acid 196 to 215 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

41. The Flavivirus according to claim 39 wherein the foreign sequence has been inserted in the region of amino acid 205 to 210 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

42. The Flavivirus according to claim 37 wherein another site comprises the region of E_0 and F_0 strands including the E_{0F0} loop which form part of the eight stranded β-barrel of domain I.

43. The Flavivirus according to claim 42 wherein the site is the loop area between E_0 and F_0 strands which form part of the eight stranded β-barrel of domain I.

44. The Flavivirus according to claim 42wherein the foreign sequence has been inserted in the region of amino acid 138 to 166 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

45. The Flavivirus according to claim 43 wherein the foreign sequence has been inserted in the region of amino acid 146 to 160 with reference to the tick-borne encephalitis virus sequence described in FIG. 2.

46. The Flavivirus according to claim 37 wherein the Flavivirus is selected from the group consisting of any Flavivirus including yellow fever virus, tick borne encephalitis virus, **dengue virus** and japanese encephalitis virus.

47. The Flavivirus according to claim 46 wherein the virus is a wild type, attenuated or recombinant virus.

48. The Flavivirus according to claims 46 and 47 wherein the Flavivirus is a yellow fever virus.

49. The Flavivirus according to claim 48 wherein the virus is a recombinant yellow fever virus.

50. The Flavivirus according to claim 49 wherein the yellow fever virus is the YF17D virus strain and substrains thereof.

51. The Flavivirus according to claims 37 to 50 wherein the foreign epitope is a malarial gene sequence.

52. A vaccine composition to immunize against flavivirus and other infectious agents consisting essentially of a virus according to claims 37 to 51.

53. The vaccine composition according to claim 52 wherein the flavivirus is a yellow fever virus and the other infectious agent is the causative agent of malaria.

54. The vaccine composition according to claim 53 wherein the malarial gene sequence is the $(NANP)_3$ humoral epitope.

55. The vaccine composition according to claim 53 wherein the malarial gene sequence is the DYENDIEKKI cytotoxic T-lymphocytes epitope.

56. The vaccine composition according to claim 53 wherein the malarial gene sequence is the SYVPSAEQI cytotoxic T-lymphocytes epitope.

57. The vaccine composition according to claims 37 to 51 comprising a sufficient amount of the virus and a pharmaceutically acceptable vehicle.

58. A Flavivirus as a vector for heterologous antigens wherein the Flavivirus is obtainable according to any of claims 1 to 19.

L17 ANSWER 12 OF 30 USPATFULL on STN
2003:250518 Recombinant dimeric envelope vaccine against flaviviral infection.
Peters, Iain D., Bozeman, MT, UNITED STATES
Coller, Beth-Ann G., Woluwe Saint Lambert, BELGIUM
McDonell, Michael, Bogart, GA, UNITED STATES
Ivy, John M., College Station, TX, UNITED STATES
Harada, Kent, Honolulu, HI, UNITED STATES
US 2003175304 A1 20030918
APPLICATION: US 2002-247960 A1 20020920 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention discloses and claims vaccines containing, as an active ingredient, a secreted recombinantly produced dimeric form of truncated flaviviral envelope protein. The vaccines are capable of eliciting the production of neutralizing antibodies against flaviviruses. The dimeric forms of truncated flaviviral envelope protein are formed 1) by directly linking two tandem copies of 80% E in a head to tail fashion via a flexible tether; 2) via the formation of a leucine zipper domain through the homodimeric association of two leucine zipper helices each fused to the carboxy terminus of an 80% E molecule; or 3) via the formation of a non-covalently associated four-helix bundle domain formed upon association of two helix-turn-helix moieties each attached to the carboxy terminus of an 80% E molecule. All products are expressed as a polyprotein including prM and the modified 80% E products are secreted from Drosophila melanogaster Schneider 2 cells using the human tissue plasminogen activator secretion signal sequence (tPA_L). Secreted products are generally more easily purified than those expressed intracellularly, facilitating vaccine production. One embodiment of the present invention is directed to a vaccine for protection of a subject against infection by **dengue virus**. The vaccine contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue virus** serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional **dengue virus** serotype dimeric E proteins similarly produced. Another embodiment of the present invention is directed to methods to utilize the dimeric form of truncated dengue envelope protein for diagnosis of infection in individuals at risk for the disease. The diagnostic contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue virus** serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The diagnostic may further contain portions of additional **dengue virus** serotype dimeric E proteins similarly produced.

CLM What is claimed:

1. A vaccine for the protection of a subject against infection by a Flavivirus, wherein said vaccine comprises a therapeutically effective amount of a dimeric 80% E, said dimeric 80% E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80% E represents the N-terminal 80% portion of the protein from residue 1 to residue 395.

2. The vaccine of claim 1 wherein said dimeric 80% E is selected from the group consisting of: linked 80% E dimer; 80% E ZipperI; 80% E ZipperII; and 80% E Bundle.

3. The vaccine of claim 2 wherein the linked 80% E dimer is a truncated envelope protein of serotype **DEN**-1.

4. The vaccine of claim 2 wherein the linked 80% E dimer is a truncated envelope protein of serotype **DEN**-2.

5. The vaccine of claim 1 wherein the linked 80% E dimer is a truncated envelope protein of serotype **DEN**-3.

6. The vaccine of claim 1 wherein the linked 80% E dimer is a truncated envelope protein of serotype **DEN**-4.

7. A **multivalent** vaccine for the protection of a subject against infection by a Flavivirus, wherein said vaccine comprises a therapeutically effective amount of a first dimeric 80% E product of one flaviviral serotype; a second dimeric 80% E product of a second flaviviral serotype; a third dimeric 80% E product of a third flaviviral serotype; and a fourth dimeric 80% E product of a fourth flaviviral serotype; wherein all dimeric 80% E products have been secreted as recombinantly produced protein from a Drosophila Schneider cell, wherein 80% E is the N-terminal 80% of the protein from residue 1 to residue 395.

8. A vaccine of claim 7 wherein said dimeric 80% E products are envelope

proteins of serotypes selected from the group consisting of: **DEN**-1; **DEN**-2; **DEN**-3; and **DEN**-4.

9. The vaccine of claim 1 wherein said Flavivirus is a **dengue virus**.

10. The vaccine of claim 2 wherein said Flavivirus is a **dengue virus**.

11. The vaccine of claim 7 wherein said Flavivirus is a **dengue virus**.

12. A method to protect a subject against a Flavivirus, which method comprises administering to a subject in need of such protection an effective amount of the vaccine of claim 1, wherein said 80% E is the N-terminal 80% of the protein from residue 1 to residue 395.

13. A method to protect a subject against a Flavivirus, which method comprises administering to a subject in need of such protection an effective amount of the vaccine of claim 1, wherein said 80% E is the N-terminal 80% of the protein from residue 1 to residue 395.

14. An immunogenic polypeptide comprising a dimeric 80% E, said dimeric 80% E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80% E represents the N-terminal 80% of the protein from residue 1 to residue 395.

15. The immunogenic polypeptide of claim 14 wherein said dimeric 80% E is selected from the group consisting of: linked 80% E dimer, 80% E ZipperI; 80% E ZipperII; and 80% E bundle.

16. The immunogenic polypeptide of claim 15 wherein the linked 80% E dimer is a truncated envelope protein which is at least one member selected from the group consisting of serotype **DEN**-1, serotype **DEN**-2, serotype **DEN**-3, and serotype **DEN**-4.

17. An immunogenic composition for the protection of a subject against infection by Flavivirus comprising the immunogenic polypeptide defined in claim 14 and a physiologically acceptable carrier.

18. The immunogenic composition defined in claim 17 further comprising an adjuvant.

19. The immunogenic polypeptide defined in claim 17 wherein said adjuvant is Iscomatrix.

20. An immunodiagnostic for the detection of Flavivirus comprising the immunogenic polypeptide defined in claim 14.

21. A **multivalent** immunodiagnostic for the detection of Flavivirus comprising at least two of the immunogenic polypeptides defined in claim 14 of at least two flaviviral serotypes.

22. A vector host recombinant DNA expression system, which comprises: a) a Drosophila host cell; b) a suitable recombinant DNA expression vector; c) DNA encoding dimeric 80% E, operably linked and under the control of a suitable promoter; and d) said DNA encoding dimeric 80% E operably linked to a secretory signal leader sequence, wherein 80% E represents the N-terminal 80% portion of the protein from residue 1 to residue 395.

23. The vector host recombinant DNA system of claim 22, wherein said dimeric 80% E is selected from the group consisting of: linked 80% E dimer; 80% E ZipperI; 80% E ZipperII; and 80% E Bundle.

24. A DNA sequence encoding the immunogenic polypeptide defined in claim 14.

25. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the immunogenic polypeptide defined in claim 14; b) a suitable support phase coated with dimeric 80% E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

26. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the **multivalent** immunodiagnostic polypeptide defined in claim 21; b) a suitable support phase coated with dimeric 80% E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

L17 ANSWER 13 OF 30 USPATFULL on STN
2003:244288 Detection and treatment of infections with immunoconjugates.

Goldenberg, David M., Medham, NJ, UNITED STATES
US 2003170697 A1 20030911
APPLICATION: US 2002-334152 A1 20021231 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of targeting a diagnostic or therapeutic agent to a focus of infection comprises injecting a patient infected with a pathogen parenterally with an antibody conjugate which specifically binds to an accessible epitope of the pathogen or of a pathogen-associated antigen accreted at the focus of infection, the antibody conjugate further comprising a bound diagnostic or therapeutic agent for detecting, imaging or treating the infection. Polyspecific composite conjugates enhance the efficacy of the method, which is especially useful for treating infections that are refractory towards systemic chemotherapy.

CLM What is claimed is:

1. A method of targeting a diagnostic or therapeutic agent to a focus of infection, which comprises injecting a patient infected with a pathogen parenterally with an antibody conjugate which specifically binds to an accessible epitope of said pathogen or of a pathogen-associated antigen accreted at said focus of infection, said antibody conjugate further comprising a bound diagnostic or therapeutic agent for detecting, imaging or treating said infection.

2. The method of claim 1, wherein said agent is a diagnostic agent selected from the group consisting of a radioisotope and a magnetic resonance image enhancing agent.

3. The method of claim 1, wherein said agent is a therapeutic radioisotope or boron addend.

4. The method of claim 1, wherein said agent is an anti-pathogenic drug or cytotoxic agent.

5. The method of claim 1, wherein said antibody conjugate specifically binds to an accessible epitope of said pathogen or pathogen-associated antigen which is not saturated or blocked by the patient's native antibodies.

6. The method of claim 5, wherein said antibody conjugate comprises a monoclonal antibody.

7. The method of claim, 1, wherein said antibody conjugate is a polyspecific conjugate which specifically binds to a plurality of accessible epitopes of said pathogen or antigen.

8. The method of claim 7, wherein said polyspecific antibody conjugate is an antiserum.

9. The method of claim 8, wherein said antiserum is affinity purified by removal of antibodies which bind to antigens associated with said pathogen circulating at a significant level in the patient's bloodstream.

10. The method of claim 8, wherein said antiserum is affinity purified by contact with bound pathogen or pathogen-associated antigens, and subsequent recovery of antiserum enriched in antibodies that bind to said pathogen or pathogen-associated antigens.

11. The method of claim 7, wherein said polyspecific conjugate is a mixture of monoclonal antibodies.

12. The method of claim 7, wherein said polyspecific conjugate is a chemically linked molecule having a plurality of antigen binding sites for said plurality of epitopes.

13. The method of claim 1, wherein said pathogen is a virus.

14. The method of claim 13, wherein said virus is an RNA virus.

15. The method of claim 13, wherein said virus is a DNA virus.

16. The method of claim 13, wherein said virus is selected from the group consisting of human immunodifficency virus (HIV), herpes virus, cytomegalovirus, rabies virus, influenza virus, hepatitis B virus, Sendai virus, feline leukemia virus, Reo virus, polio virus, human serum parvo-like virus, simian virus 40, respiratory syncytial virus, mouse mammary tumor virus, Varicella-Zoster virus, **Dengue virus**, rubella virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukiemia virus, mumps virus, vesicular

stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus and blue tongue virus.

17. The method of claim 1, wherein said pathogen is a bacterium.

18. The method of claim 17, wherein said bacterium is selected from the group consisting of Streptococcus agalactiae, Legionella pneumophilia, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhosae, Neisseria meningitidis, Pneumococcus, Hemophilis influenzae-B, Treponema pallidum, Lyme disease spirochetes, Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus, Mycobacterium tuberculosis and Tetanus toxin.

19. The method of claim 1, wherein said pathogen is a protozoan.

20. The method of claim 19, wherein said protozoan is selected from the group consisting of Plasmodium falciparum, Plasmodium vivax, Toxoplasma gondii, Trypanosoma rangeli, Trypanosoma cruzi, Trypanosoma rhodesiensei, Trypanosoma brucei, Schistosoma mansoni, Schistosoma japanicum, Babesia bovis, Elmeria tenella, Onchocerca volvulus, Leishmania tropica, Trichinella spiralis, Onchocerca volvulus, Theileria parva, Taenia hydatigena, Taenia ovis, Taenia saginata, Echinococcus granulosus and Mesocestoides corti.

21. The method of claim 1, wherein pathogen is a helminth.

22. The method of claim 1, wherein said pathogen is mycoplasma.

23. The method of claim 22, wherein said mycoplasma is selected from the group consisting of Mycoplasma arthritidis, M. hyorhinis, M. orale,. M. arginini, Acholeplasma laidlawii, M. salivarium and M. pneumoniae.

24. The method of claim 1, which further comprises administering to said patient, at a time after administration of said conjugate sufficient to optimize uptake of said conjugate at the site of said infection, an amount of a second antibody that specifically binds to said conjugate sufficient to reduce the amount of said conjugate in circulation by 10-85% within 2-72 hours.

25. The method of claim 1, wherein said agent is a therapeutic antibiotic or cytotoxic agent that causes hematopoietic toxicity as a side effect of its administration, and wherein said method further comprises administering to said patient, at a time prior to, concomitant with or subsequent to administration of said therapeutic conjugate, an amount of a cytokine sufficient to mitigate or prevent the hemtopoietic toxicity of said agent.

26. An antibody conjugate for targeting a focus of infection, comprising an antibody or antibody fragment which binds to an epitope on a single species of pathogen or an antigen derived therefrom, said antibody or antibody fragment being conjugated to at least one diagnostic or therapeutic agent.

27. An antibody conjugate for targeting a focus of infection, comprising an immunoreactive composite of a plurality of chemically linked antibodies or antibody fragments which bind to a plurality of epitopes on a single species of pathogen or an antigen derived therefrom, said composite being conjugated to at least one diagnostic or therapeutic agent.

28. A sterile injectable preparation for targeting a focus of infection in a human patient, comprising the antibody conjugate of claim 26, in combination with a pharmacologically acceptable sterile injection vehicle.

29. A kit for use in preparing a sterile injectable preparation for targeting a focus of infection in a human patient, comprising in suitable containers, the antibody conjugate of claim 26 and a pharmacologically acceptable sterile injection vehicle.

L17 ANSWER 14 OF 30 USPATFULL on STN
2003:155723 Polynucleotides encoding flavivirus and alphavirus **multivalent** antigenic polypeptides.
Punnonen, Juha, Palo Alto, CA, United States
Bass, Steven H., Hillsborough, CA, United States
Whalen, Robert Gerald, Paris, FRANCE
Howard, Russell, Los Altos Hills, CA, United States
Stemmer, Willem P. C., Los Gatos, CA, United States

Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
US 6576757 B1 20030610
APPLICATION: US 2000-724852 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed to antigen library immunization, which provides methods for obtaining antigens having improved properties for therapeutic and other uses. The methods are useful for obtaining improved antigens that can induce an immune response against pathogens, cancer, and other conditions, as well as antigens that are effective in modulating allergy, inflammatory and autoimmune diseases.

CLM What is claimed is:

1. A nucleic acid comprising a polynucleotide sequence encoding a recombinant **multivalent** antigenic polypeptide that comprises multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant **multivalent** antigenic polypeptide induces an immune response against the first and second antigenic polypeptides that is greater than the immune response induced by any one of the fist and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

2. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

3. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a **Dengue virus**, a yellow fever virus, a St. Louis encephalitis virus, and a Murray Valley encephalitis virus, Kunjin virus, and West Nile virus.

4. The nucleic acid of claim 1, wherein each of at least the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

5. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are different serotypes of a flavivirus or alphavirus.

6. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are different species or strains of a flavivirus or alphavirus.

7. A vector comprising the nucleic acid of claim 1.

8. The vector of claim 7, wherein the vector comprises an expression vector.

9. A host cell comprising the nucleic acid of claim 1.

10. A host cell comprising the vector of claim 7.

11. The host cell of claim 9, wherein the host cell is in vivo.

12. The host cell of claim 9, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

13. A method of producing a recombinant **multivalent** antigenic polypeptide comprising culturing a host cell comprising the expression vector of claim 8 under conditions suitable for expression of the **multivalent** antigenic polypeptide.

14. The method of claim 13, further comprising isolating the **multivalent** antigenic polypeptide.

15. A composition comprising the nucleic acid of claim 1 and an excipient.

16. The nucleic acid of claim 1, wherein the **multivalent** antigenic

polypeptide induces an immune response to the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any of the first and second antigenic polypeptides.

17. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least the first and second antigenic polypeptides and at least a third antigenic polypeptide of a flavivirus or alphavirus.

18. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least two different serotypes, strains, or species of a flavivirus or alphavirus.

19. The nucleic acid of claim 18, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least three different serotypes, strains, or species of a flavivirus or alphavirus.

20. The nucleic acid of claim 19, wherein the **multivalent** antigenic polypeptide induces an immune response against a disease condition caused by one or more of at least three different serotypes, strains, or species.

21. A nucleic acid which encodes a recombinant **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant **multivalent** antigenic polypeptide is prepared by a method comprising: (1) recombining at least a first nucleic acid comprising a nucleotide sequence that encodes the first antigenic polypeptide and at least a second nucleic acid comprising a nucleotide sequence that encodes the second antigenic polypeptide, wherein at least the first and second nucleic acids differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library of recombinant nucleic acids for at least one recombinant nucleic acid that encodes a recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

22. The nucleic acid of claim 21, wherein the method further comprises: (3) recombining at least one recombinant nucleic acid with at least a third nucleic acid comprising a nucleotide sequence that encodes a third antigenic polypeptide of a third flavivirus or alphavirus, wherein the third nucleic acid is the same or different from at least the first and second nucleic acids, to produce a further library of recombinant nucleic acids; (4) screening the further library of recombinant nucleic acids for at least one further recombinant nucleic acid that encodes a recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus; and (5) repeating (3) and (4), as necessary, for a further recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus.

23. The nucleic acid of claim 21, wherein at least the first and second antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.

24. The nucleic acid of claim 22, wherein at least first, second, and third antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.